STUDIES IN

PHYSIOLOGICAL UNDERNOURISHMENT

IN SHEEP

by

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I - INTRODUCTION

The nutritional state of an animal is dependent on the supply of and demand for nutrients. In situations in which the intake of nutrients does not meet requirements, the animal is described as undernourished, and its nutritional state as one of undernourishment. Undernourishment does not necessarily imply a low level of nutrient intake, and can occur in an animal on what might be generally considered as a 'high plane of nutrition' if the nutrient requirements of that animal are, for one of a variety of reasons, unusually high.

Blaxter (1957) has stated that the principal nutritional factor limiting hill sheep production is the supply of available energy. This is likely to be particularly true during the pregnancy period when the intake of energy is low and the requirement for energy high. There is, however, little factual information in the literature regarding the extent to which the supply of available energy fails to meet the requirements of the pregnant hill ewe, or of the consequences of this presumed undernourishment on subsequent production. The overall objective of this thesis is to provide factual information regarding the extent and effects of the energy deficiency experienced by hill ewes during pregnancy.

The experimental work reported deals firstly with the quantitative changes in body weight and composition of free-grazing pregnant hill ewes, and with the physiological characterization of the undernourishment indicated by these data. Consideration is given to the relative importance of the principal factors determining the severity of undernourishment in individual pregnant ewes, and to the choice of parameters by which this undernourishment may be most

efficiently measured. Conclusions drawn from these studies led to further work in which certain biochemical parameters were used as a means of controlling nutritional state in an experiment designed to determine the effect of undernourishment during pregnancy on lamb birth-weight and to assess the energy requirements of the pregnant ewe.

The results of the experimental work are discussed in relation to existing knowledge, and consideration is also given to the theoretical and practical implications of certain experimental findings.

II - REVIEW OF LITERATURE

In the first three sections of this chapter the literature relevant to the title and context of this thesis is reviewed. Only those contributions having a direct bearing on the experimental work presented in a later chapter have been considered at this stage. The final two sections, which deal with certain aspects of ruminant physiology and metabolism, are more in the nature of preliminary considerations than comprehensive reviews of existing knowledge; certain biochemical parameters have been used in the experimental work as means to an end and not as ends in themselves, and these sections have been included in order that the use of these parameters may be considered in their proper physiological perspective, and not in isolation.

1. Annual Changes in Live Weight and Body Composition

It is widely recognised that pregnancy, in the free-grazing hill ewe, constitutes a period of high energy demand and low energy intake. Although this fact and some of its consequences are regarded as common knowledge (Fraser, 1939), there is little factual information in the literature regarding the pattern or extent of the annual cycle of live weight found in hill ewes, and almost no information on body composition or components of live-weight loss relevant to the hill situation.

Robinson, Currie and Peart (1961) noted losses ranging from 4 to 20% and averaging 16 to 17% of maximum live weight in traditionally managed Scottish Blackface ewe flocks. Their data were based on infrequent weighings (four or five per year) and estimates of

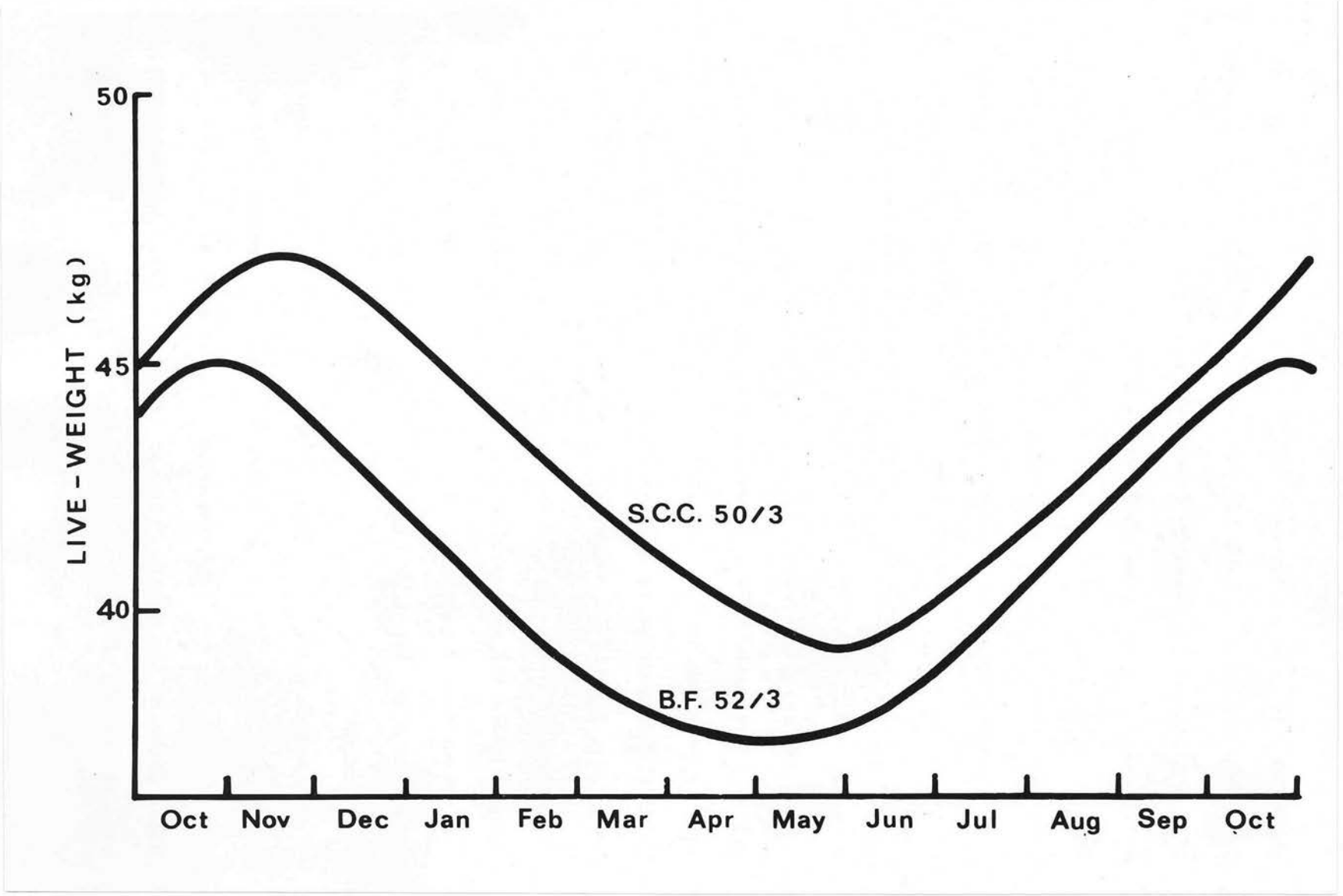

Fig. 1. Annual changes in mean live weight in a South Country Cheviot ewe flock over the years 1950–1953, and in a Scottish Blackface ewe flock during the year 1952–1953, computed from monthly records of live weight. (Hill Farming Research Organisation, unpublished report.)

post-parturient live weight, and as such are of limited value in characterizing the pattern of live-weight change.

More detailed information is contained in unpublished reports of the Hill Farming Research Organisation. Monthly records of live weight were obtained from three hefts of South Country Cheviot ewes from autumn 1950 to spring 1953, and two hefts of Scottish Blackface ewes during twelve months from February 1952. Mean annual live-weight curves have been prepared from these data and are presented in Figure 1. Live weight was at a maximum in late autumn and declined progressively from before mating in late November-early December until about the time of parturition or early lactation. During and after lactation, live weight increased until the maximum was again reached in late autumn. The extent of the live-weight loss (approximately 18%), as estimated from the curves in Figure 1, is of the same order as that reported by Robinson et al (1961). These data and those of Robinson et al may, however, underestimate the extent of the weight loss. Lambing is spread over a period of several weeks and, as neither set of data contains accurate post-parturient live weights, it is likely that individual live-weight losses were greater than the mean figures for pregnant and lactating ewes suggest.

Although little published information on body composition is available, it is generally considered that free-grazing hill ewes in this country are less fat than those of other breeds in this and other parts of the world. Fraser (1960) has described Scottish hill ewes at the end of winter as being "pregnant skeletons concealed in wool". Reports on the composition of changes in ewe live weight

have generally been concerned with fat or over-fat ewes (e.g. Kirton and Barton, 1958a, 1958b; Panaretto, 1964; Hight and Barton, 1965) and consideration of the results of these studies is more appropriate to the later discussion of experimental results than in the present context.

2. Effect of Nutrition During Pregnancy on Lamb Birth-Weight

Evidence reviewed by Thomson and Aitken (1959) and the results of more recent studies (e.g. Wallace, 1961; Coop, 1962a, 1964; Bennett, Axelsen and Chapman, 1964) show clearly that the level of energy intake during the weeks immediately before and after mating is of importance in determining the number of lambs born. It is generally considered, however, that the level of nutrition of the ewe during early pregnancy (i.e. up to 90 days) has no effect on lamb birth-weight, except in so far as nutrition during this period may affect the number of multiple births. Early studies by Thomson and Fraser (1939), Wallace (1948a) and Gill and Thomson (1954a, 1954b), and more recent work by Hodge (1966) failed to show an effect on birth-weight of restricted levels of intake during the first 90 days of pregnancy. Everitt (1964), however, noted that Merino ewes, which lost 12% gross body weight during the first 90 days of pregnancy, had significantly lighter foetuses and functional cotyledon material than ewes which gained 12% during the same period, and Bennett et al (1964), Taplin and Everitt (1964), and Everitt (1966, 1967) found that effects of maternal undernutrition during the first three months of pregnancy were reflected in lower birth-weights. McClymont and Lambourne (1958) also noted an effect of restricted maternal nutrition during the first four months of pregnancy on birth-weight.

It is difficult to find a single factor which can reconcile these apparently anomalous results. The majority of the nutritional treatments in these experiments were achieved by grazing management practices, and precise nutritional data are not available for comparison. Although approximately proportional live-weight changes were noted in experiments in which birth-weight differences resulting from early nutritional restriction were and were not found, there is inadequate information regarding patterns of live-weight change and initial condition of the ewes to assess satisfactorily the relative degrees of undernourishment in the several investigations. Although the possibility that significant effects were obtained by more severe undernourishment at a particular, but unknown, critical time cannot be excluded, it is noteworthy that in Bennett et al's (1964) study there was an appreciably greater reduction in the birth-weights of single lambs as a result of early nutritional restriction in two-year old Merino ewes than in older crossbreds (24% and 4% respectively). The age difference may not be without significance, but it is more probable that the anomalous results are due to a genetic difference between breeds. The results noted by Everitt (1964, 1966, 1967) and Taplin and Everitt (1964) were obtained using three-year old Merino ewes; those workers who failed to detect a significant effect on birth-weight of nutritional restriction during the first 90 days of pregnancy worked with Scottish Halfbred (Thomson and Fraser, 1939; Wallace, 1948a; and Gill and Thomson, 1954a, 1954b) and Merino Crossbred (Hodge, 1966) ewes.

Approximately 70% of foetal growth takes place during the final 30% of gestation (Winters and Feuffel, 1936), and it is generally

accepted that the level of energy intake during the later stages of pregnancy (usually regarded as 90 to 140 days) has a greater effect on lamb birth-weight than that in the earlier period. The available information relating maternal nutrition during late pregnancy to lamb birth-weight is summarized in Table 1, which is an extension of reviews by Thomson and Aitken (1959) and Schinckel (1963). Change in maternal body-weight is used in this table as an index of level of nutrition in an attempt to find a common factor on which comparisons may be made between experiments with broadly similar objectives, but carried out under a wide variety of conditions and involving different breeds, levels of nutrition and techniques of feeding.

The data in Table 1 indicate a dependence of lamb birth-weight on maternal body-weight change during the later stages of pregnancy. In general, it will be noted that ewes which gained most weight during the final two months of pregnancy gave birth to heavier lambs than ewes which gained less, or actually lost, weight. It is also clear that, in the majority of instances, restricted maternal nutrition had a disproportionately large effect on the birth-weight of twin lambs. In those experiments in which there was little or no evidence of an effect of maternal nutrition on birth-weight, the changes in ewe body weight suggest either that the differences in levels of intake between treatment groups were relatively small (e.g. Coop, 1950; Guyer and Dyer, 1954), or that the "low" level of nutrition was in fact relatively high (cf. Papadopoulos and Robinson, 1957, and Schinckel and Short, 1961). Jefferies and Fearn (1956) offer no explanation of their somewhat surprising finding that ewes grazing lucerne and receiving high quality supplementary feeding

TABLE I

Effect on lamb birth-weight of nutritionally induced changes in ewe body weight during pregnancy

Authors and Reference	Breed of ewe	Change in ewe weight (kg)	Period of weight change (weeks)	Lamb birth-weights		Differences in birth weights	
				Singles (kg)	Twins (kg)	Singles (kg)	Twins (kg)
Vergés (1939) Pálsson & Vergés (1952)	Scottish Half Bred	+ 18.0 + 0.5	6 6		4.1 2.8		1.3
Underwood, Shier & Cariss (1943)	Merino	+ 10.5 0 to + 2.3	4-6 4-6	4.8 4.4	4.3 3.1	0.4	1.2
		+ 4.1 - 0.5	4-6 4-6	4.8 4.0	4.3 3.4	0.8	0.9
Wallace (1948a)	Scottish Half Bred	+ 14.0 + 0.5 - 6.5	6 6 6		5.0 3.5 2.6		1.5 0.9
		+ 18.0 - 4.7	6 6		4.7 3.2		1.5

TABLE I (cont'd.)

Authors and Reference	Breed of ewe	Change in ewe weight (kg)	Period of weight change (weeks)	Lamb birth-weights		Difference in birth weights	
				Singles (kg)	Twins (kg)	Singles (kg)	Twins (kg)
Barnicoat, Logan & Grant (1949)	Romney	+ 8.0 + 2.7	6 6	4.7 4.4	3.8 3.4	0.3	0.4
Thomson & Thomson (1949, 1953)	Cheviot	+ 13.5 - 2.2	10 10	4.8 3.7	3.5 2.3	1.1	1.2
Coop (1950)	Corriedale	+ 12.5 + 3.0	20 20	4.6 4.3	3.8 3.7	0.3	0.1
		+ 9.5 - 1.8	20 20	4.3 4.2	3.8 3.2	0.1	0.6
Thompson (1950)	Dorset Horn - Merino	+ 5.0 - 5.0	7 7	4.3 3.9	3.4 3.1	0.4	0.3

TABLE I (cont'd.)

Authors and Reference	Breed of ewe	Change in ewe weight (kg)	Period of weight change (weeks)	Lamb birth-weights		Difference in birth weights	
				Singles (kg)	Twins (kg)	Singles (kg)	Twins (kg)
Gill & Thomson (1954a, 1954b)	Scottish Half Bred	+ 14.5 + 11.8 + 5.9 + 2.7	11 11 11 11		5.3 5.1 4.8 4.3		0.2 0.3 0.5
		+ 9.1 + 6.8 - 1.0	11 11 11		5.0 4.9 3.8		0.1 1.1
Guyer & Dyer (1954)	North-western	+ 9.1 + 1.4	8 8	5.1 4.7		0.4	
		+ 11.0 + 4.8	8 8	5.5 5.4		0.1	
		+ 10.6 + 2.7	8 8		4.1 3.4		0.7
		+ 11.5 + 5.9	8 8		4.4 4.1		0.3

TABLE I (cont'd.)

Authors and Reference	Breed of ewe	Change in ewe weight (kg)	Period of weight change (weeks)	Lamb birth-weights		Difference in birth weights	
				Singles (kg)	Twins (kg)	Singles (kg)	Twins (kg)
Jefferies & Fearn (1956)	Merino ?	High (5 kg difference		3.7	3.1	0.7	0.4
		Low (4 weeks before lambing		4.4	3.5		
Papadopoulos & Robinson (1957)	Romney x Merino	+ 24.5	13	5.4	4.6	0	0
	B.L. x Merino	+ 9.1	13	5.4	4.6		
Schinckel & Short (1961)	Merino	+ 10	20	3.9		1.3	
		- 10	20	2.6			

TABLE I (cont'd.)

Authors and Reference	Breed of ewe	Change in ewe weight (kg)	Period of weight change (weeks)	Lamb birth-weights		Difference in birth weights	
				Singles (kg)	Twins (kg)	Singles (kg)	Twins (kg)
Gardner & Hogue (1963)	Western White-faced	+ 4.4 + 1.7	6 6	4.7 4.7		0	
		+ 2.8 - 0.7 - 2.7	6 6 6	5.1 5.0 4.9		0.1 0.1	
		+ 2.6 - 1.7	6 6		4.0 3.6		0.4
		- 0.4 - 4.8 - 7.3	6 6 6		4.5 4.1 3.7		0.4 0.4
Forbes & Robinson (1966)	Greyface	+ 10.2 + 7.3	8 8		4.8 3.9		0.9
		+ 10.9 + 6.2	8 8		4.6 4.0		0.6

gave birth to lighter lambs than less heavy ewes grazing unimproved pasture and receiving a low quality supplement. All ewes were reported to be in very fat condition, and it is possible that a reduction in voluntary feed intake, similar to that noted by Reid and Hinks (1962a) and Everitt (1966) in fat pregnant ewes, was responsible for the lower birth-weights from the heavier ewes.

3. Energy Requirements During Pregnancy

There are few reports in the literature concerning the energy requirements of the pregnant ewe. The results of experiments reviewed in the preceding section make a valuable contribution to knowledge by establishing the effects of certain changes in gross maternal live weight at different stages of pregnancy on subsequent lamb birth-weight, but the majority of these experiments were conducted under field or group-feeding conditions without close control or measurement of individual intakes, and as such make little contribution regarding actual energy requirements. There is no reason to assume that in those experiments in which significant effects on birth-weight were recorded, the higher levels of nutrition were optimum levels, and that further increases in intake would not have resulted in even higher birth-weights; if the foetus represents approximately 60% of the weight loss at parturition (Winters and Feuffel, 1936) it is evident that in several of the reports summarized in Table 1 the higher levels of intake must have resulted in a net loss in weight of maternal tissue (e.g. Gill and Thomson, 1954a, 1954b). Conversely, it is probable that in other experiments (e.g. Papadopoulus and Robinson, 1957) the lower level of nutrition was more than adequate for maximum foetal growth.

Thomson and Aitken (1959) considered that in the ideal situation "the ewe should gain sufficient weight to allow optimum development of the genital tract and contents and of the udder, without loss of weight in other tissues". Estimates of the weights of these products of conception are contained in the reports of Cloete (1939) and Wallace (1948b) who measured increases in weight of mammary tissue and the gravid uterus at different stages of pregnancy. Using Cloete's data, Thomson and Aitken (1959) calculated that, in a Merino ewe weighing approximately 40 kg at mating and carrying a single foetus weighing 4 kg at term, a daily live-weight increase of 0.15 kg would be necessary during the final month of pregnancy to prevent loss of weight from maternal tissues. Similar calculations based on Wallace's data indicated that daily gains of 0.3 and 0.4 kg respectively would be required to prevent loss from maternal tissues during late pregnancy in 60 kg single- and twin-bearing Scottish Halfbred ewes. Thomson and Aitken considered these calculations in relation to the results of Coop (1950), Guyer and Dyer (1954) and Papadopoulus and Robinson (1959) and concluded that "live-weight gains in late pregnancy, smaller than the expected gains of gravid uterus and udder, are consistent with good reproductive performance". Gains of 6 to 7 kg during the last eight weeks of pregnancy in ewes carrying single foetuses of about 5 kg were considered to be adequate.

At the time Thomson and Aitken (1959) published their review there were virtually no reports based on critical experimentation regarding the levels of nutrition required to produce these prescribed live-weight changes. Six years later a publication of the Agricultural Research Council (1965) on the nutrient requirements of

ruminants listed the energy requirements of the pregnant ewe as "not available". There are, however, reports by the United States National Research Council Committee on Animal Nutrition (1957, 1964), Reid and Hinks (1962a) and Reid (1963) which provide estimates of energy requirements during pregnancy, and reports by Whiting and Slen (1958), Smoliak and Slen (1958), Wright, Pope and Phillips (1962) and Gardner and Hogue (1963) concerning the adequacy of the National Research Council recommendations.

The National Research Council (1957, 1964) give a figure of 1.8 lb total digestible nutrients (TDN) per 100 lb live weight as the energy requirement of the ewe during the final six weeks of gestation. Although there is a certain amount of disagreement in the literature regarding the conversion of TDN values to units of energy (Brody, 1945; Swift, 1957; Garrett, Meyer and Lofgreen, 1959; Coop, 1962b; Coop and Hill, 1962; Lambourne and Reardon, 1963), the most commonly used factor of 1 lb TDN = 1616 kcal metabolizable energy (ME) gives a value for the National Research Council requirement of 64 kcal ME per kg live weight. If an allowance of 33 kcal ME per kg is subtracted for the cost of maintenance (Coop, 1962b; Lambourne and Reardon, 1963; Langlands, Corbett, McDonald and Pullar, 1963a, 1963b), the energy available to the foetal tissues is 31 kcal per kg ewe body weight. To convert this value to energy requirements per unit weight of foetus it is necessary to ascribe certain arbitrary weights to ewe and foetus. For example, for a 45 kg ewe with a 4.5 kg single foetus (the "average" Scottish Blackface ewe) the energy requirement of the foetus would be 310 kcal ME per kg.

Reid and Hinks (1962a) noted that increased energy requirements in late pregnancy were correlated with foetal weight, and calculated

the additional daily feed requirements per unit weight of foetus. This was 150 g of a 1 : 1 mixture of chaffed wheaten and lucerne hays per kg foetus, equivalent to 320 kcal ME (Reid, 1963). Assuming a ewe body weight of 55 kg and a lamb birth-weight of 6 kg (these values estimated from graphs presented by Reid and Hinks) the value of 31 kcal ME per kg ewe body weight calculated from the National Research Council figure gives a requirement for foetal tissues of 285 kcal per kg foetus, which is in reasonable agreement with the published findings of Reid (1963) quoted above. In making these calculations and comparisons it must be borne in mind that the National Research Council (1957, 1964) recommendation is based on ewe body weight, and refers to the final six-week period as a whole. The figure computed by Reid (1963) is based on foetal weight, which is increasing rapidly during the final weeks of pregnancy, and refers to the extra energy required to prevent maternal undernourishment up to the time of parturition.

The National Research Council (1957) recommendation made no allowance for differences in foetal weight. Subsequent studies by Whiting and Slen (1958), Smoliak and Slen (1958), Wright, Pope and Phillips (1962) and Gardner and Hogue (1963) indicated that although the recommended value of 1.8 lb TDN per 100 lb live weight was sufficient for single-bearing ewes during late pregnancy, it was probably inadequate for ewes with twin foetuses. The TDN figure remains unchanged in the revised nutrient requirements (National Research Council, 1964), but the higher energy intake required by ewes producing twin lambs is recognized in the text. Gardner and Hogue (1963) estimated this higher requirement as 125% of the recommended figure.

4. Metabolism of the End-products of Ruminant Digestion

This particular section is not intended as a comprehensive review of the literature dealing with ruminant digestion and metabolism, but is included as a means of presenting basic information regarding the origin of certain circulating metabolites, before discussing the use of these metabolites as parameters in the measurement and control of nutritional state.

It has been known since late last century that the fermentation of complex carbohydrates in the rumen produces large amounts of volatile fatty acids (VFA). The full significance of this discovery was not, however, appreciated until some twenty-five years ago, when Barcroft and his colleagues demonstrated that a large proportion of the energy available to ruminants was derived from the metabolism of these acids (principally acetic, propionic and butyric). McAnally and Phillipson (1942) and Barcroft, McAnally and Phillipson (1944) showed that VFA's produced in the rumen, as a result of microbial fermentation, were also absorbed from that organ and metabolized in the liver and other tissues. It is now known that the pattern of production and subsequent absorption of the individual VFA's is dependent on diet, ration, and feeding regime (see reviews by Annison and Lewis, 1959; Barnett and Reid, 1961; Blaxter, 1961, 1962; Annison, 1965).

A recent review by Reid (1968) indicates that over a wide range of diets the rate of total VFA production is of the order of 1 to 5 moles per 24 hr. Assessment of production rates of individual acids is complicated. Unless animals are in a steady state, rumen concentrations of individual acids at a particular point in time mean

little (Ulyatt, 1967); *in vitro* estimates of production rates invariably give lower results than *in vivo* techniques (Faichney, personal communication). In experiments using infusions of C^{14}-labelled acids, interpretation is made difficult by interconversions between acids, particularly acetic and butyric (Weller, Gray, Pilgrim and Jones, 1967). Examples calculated from results of three recent experiments based on the infusion of C^{14}-labelled acids are presented in Table 2, and give an indication of net production rates of individual acids from a limited range of diets. Examples of more extreme molar proportions of the principal VFA resulting from the fermentation of a wider variety of foodstuffs have been given by Annison and Lewis (1959) and Blaxter (1962). Estimates of the contribution of energy from VFA to total digestible energy in the experiments referred to in Table 2 range from approximately 50 to over 60%. Contributions to digestible energy from branched chain and higher VFA produced in the rumen are considered to be negligible, and those from VFA production from food residues in the caecum and colon are unlikely to exceed 5% (Hungate, Phillips, McGregor, Hungate and Buechner, 1959).

There is no evidence of active transport of VFA across the rumen epithelium, and it appears that concentration gradient is the most important factor in determining rates of transfer (Annison, 1965). Acetate and propionate are absorbed from the rumen and reach the liver as such, but in most situations at least the greater part of the butyrate is metabolized by the rumen epithelium and converted to ketone bodies (acetoacetic and β-hydroxybutyric acids) (Annison, Hill and Lewis, 1957; Annison and Lindsay, 1962; Roe, Bergman and Kon, 1966). Techniques used in measuring absorption rates of these

TABLE 2

Examples of Net VFA Production Rates from Different Feeds
(calculated from results presented in papers quoted below)

Authors and Reference	Ration	Diet	Net VFA Production Rate (moles/24 hr)		
			Acetic	Propionic	Butyric
Bergman, Reid, Murray, Brockway and Whitelaw (1965)	900 g	dried grass	3.7	1.0	0.7
Leng and Brett (1966)	800 g	lucerne chaff	5.3	1.4	0.4
	400 g 200 g	maize lucerne chaff	3.7	1.1	0.5
	300 g 300 g	maize lucerne chaff	3.3	0.8	0.4
	450 g 50 g	wheaten straw lucerne	1.8	0.6	0.2
Gray, Weller, Pilgrim and Jones (1967)	500 g 500 g	wheaten hay lucerne hay	2.8	0.8	0.5
	400 g 600 g	wheaten day lucerne hay	3.3	0.8	0.6
	1000 g	lucerne hay	2.9	0.7	0.5

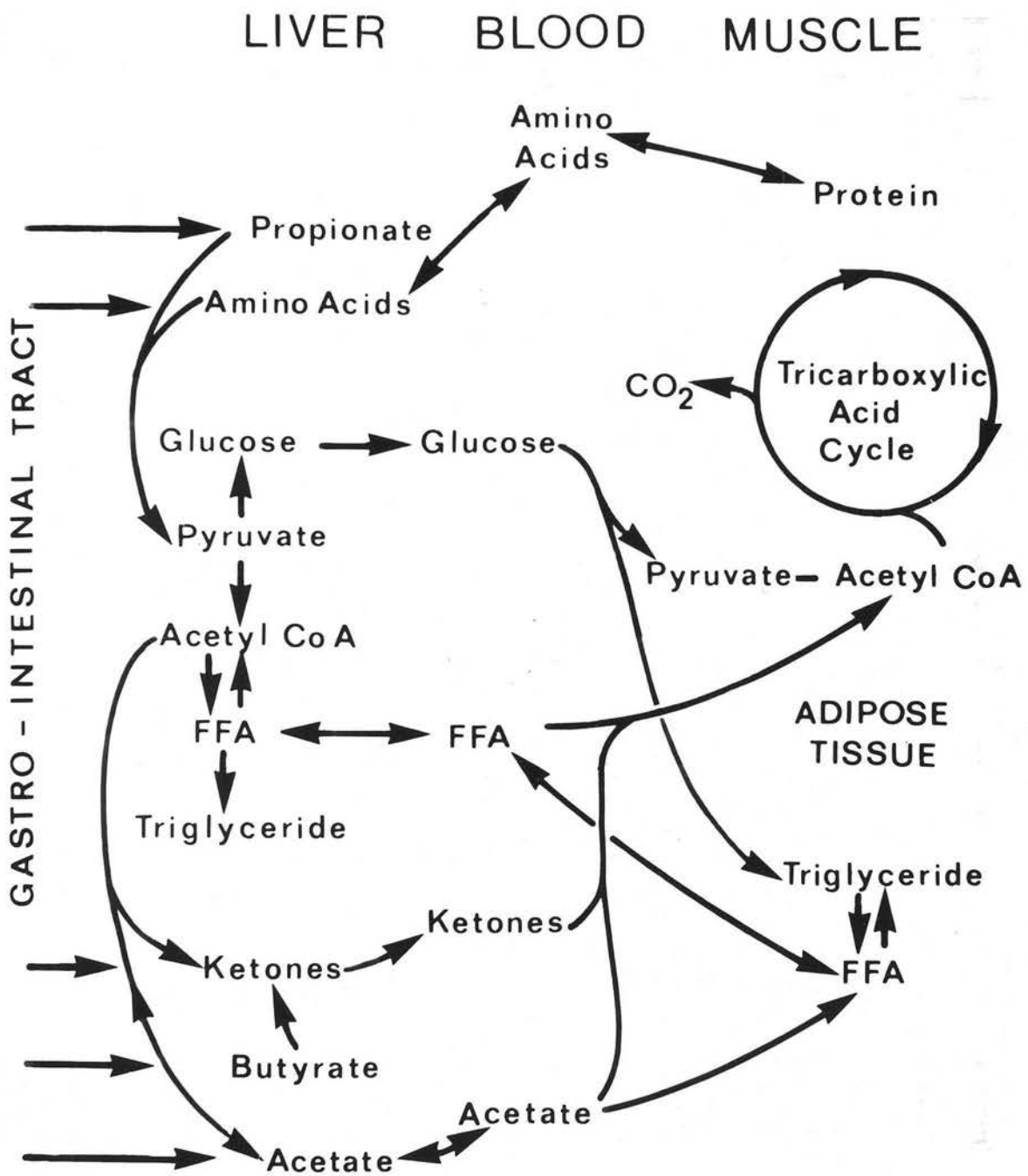

Fig. 2. Principal pathways by which the end-products of ruminant digestion are metabolized (after Reid, 1968).

metabolites have been discussed by Blaxter (1962) and Annison (1965). Reid (1968) calculated that, in a 50 kg sheep fed a predominantly roughage diet at a maintenance level, 3.5 moles acetic acid, somewhat less than 1.0 mole propionic acid, and 0.5 mole ketone bodies reach the liver every 24 hr from the gastrointestinal tract. These metabolites account for approximately 70% of the digestible energy, the majority of the remaining 30% coming from absorbed amino acids (Blaxter, 1962; Reid, 1968).

The pathways by which these end-products of ruminant digestion are metabolized are well established and have been discussed in detail in reviews (Annison and Lewis, 1959; Blaxter, 1962) and in the published proceedings of specialist conferences (Lewis, 1961; Armstrong, 1965a; Blaxter, 1965; Dougherty, Allen, Burroughs, Jacobson and McGilliard, 1965). Particular pathways of relevance to experimental work described in later sections are illustrated in Figure 2, which has been taken from Reid (1968).

Only a very small proportion of absorbed acetate is converted to ketone bodies in the liver, most of it passing into the peripheral circulation where it is rapidly metabolized via acetyl-CoA and the tricarboxylic acid cycle in extrahepatic tissues. Acetate is used for energy production by skeletal muscle, heart and kidney, and probably also for fat synthesis in adipose tissue (Armstrong, 1965b; Reid, 1968).

Absorbed propionate reaches the liver via the portal vein and is rapidly converted to glucose through the tricarboxylic acid cycle in that tissue. Although the importance of propionate as a major substrate for gluconeogenesis has been appreciated for some time

(Annison et al, 1957), there has been some uncertainty regarding the precise quantitative conversion of propionate to glucose. A review by Lindsay (1959a) concluded that propionate alone could not meet the demands for gluconeogenesis; evidence from an experiment by Bergman, Roe and Kon (1966) indicated that in a normal animal only 20 to 40% (depending on diet) of glucose was derived from propionate, and that the greater part of the remainder was accounted for by protein. These workers estimated that only 50% of propionate was converted to glucose, and suggested that the remainder probably spared the use of protein and other glucogenic substrates for non-glucose purposes, thus contributing indirectly to net gluconeogenesis. More recently Leng, Steel and Luick (1967) have shown that considerable amounts of propionate are first converted to lactate, and that the net contribution of propionate to gluconeogenesis is likely to be appreciably greater than the results of earlier experiments had suggested.

Any butyrate not metabolized by the rumen epithelium is rapidly converted to β-hydroxybutyrate in the liver, prior to oxidation by heart, kidney and muscular tissue. Although administration of butyrate or β-hydroxybutrate causes an increase in blood glucose concentration (Sutherland, 1963), experiments by Annison, Leng, Lindsay and White (1963) have shown that this effect is not due to the synthesis of glucose from ketone bodies. Armstrong (1965b) has suggested that this result may be due in part to a glucose-sparing effect of β-hydroxybutyrate, and in part to a stimulating effect of acetoacetate (formed from β-hydroxybutyrate) on gluconeogenesis from lactate. Normal pre-feeding plasma ketone concentrations of less than 2 mg % (expressed as acetone) may increase to 3 to 4 mg % after feeding as a result of the metabolism of butyrate in the rumen epithelium.

The supply of energy to the tissues varies throughout the day according to the level and pattern of the nutrient intake. Concentrations of blood acetate are sensitive to the pattern of intake and may vary from pre-feeding levels of 2 to 6 mg % (milligrams per 100 millilitres) to post-feeding concentrations of 8 to 25 mg %. The rate of acetate utilization by the tissues is proportional to the arterial concentration (Reid, 1950). In contrast to acetate, blood glucose concentrations show relatively little response to the pattern of feeding, or even to relatively large changes in nutrient intake; normal pre-feeding blood glucose levels of 35 to 45 mg % (equivalent to plasma concentrations of 55 to 65 mg %) may increase by 10 to 20 mg % after feeding, particularly if the feed is eaten quickly (Reid and Hinks, 1962c). The rate of glucose utilization is controlled by insulin, and increases markedly as plasma glucose levels rise; Reid (1968) quotes an example of a utilization rate of 90 g per 24 hr increasing to 130 g per 24 hr following a post-prandial increase in plasma glucose concentration of 20 mg %. Recent work by Manns and Boda (1967) and Manns, Boda and Willes (1967) indicates that propionate and butyrate may be implicated in the control of insulin secretion, and thus have an effect on glucose utilization rate.

Circulating concentrations of acetate and glucose during the first few hours after feeding are generally in excess of tissue requirements, and the surplus is available for the synthesis of higher fatty acids in adipose tissue. As the availability of these fat precursors declines with increasing time from feeding, stored fat is progressively mobilized in the form of free or non-esterified fatty acids (FFA or NEFA). The normal range of plasma FFA

concentrations in an animal on a maintenance level of feeding is from 100 to 200 μeq/l (microequivalents per litre) several hours after feeding to 500 to 600 μeq/l immediately before feeding (Annison, 1960; Reid and Hinks, 1962c). This diurnal variation is most marked in animals consuming a single daily feed in a short time, and is likely to be least in grazing animals in which the intake is more evenly spread over the 24 hr period. Results of experiments by Radloff, Schultz and Hoekstra (1966) on cows and goats indicate that diurnal variations and other responses of plasma FFA to nutritional changes are greater in individuals which are lactating.

5. Measurement and Control of Nutritional State

The effect of a moderate reduction in nutrient intake, from a maintenance or higher level to a submaintenance level, is to move the lipogenesis-lipolysis equilibrium towards increased lipolysis. This is reflected in higher pre-feeding plasma FFA levels and in an increased diurnal variation in plasma FFA concentration, but may not necessarily involve any significant change in plasma glucose or ketone concentrations (Reid and Hinks, 1962c), although the absorption of the precursors of these metabolites must be reduced.

More severe undernourishment, such as that caused by continued fasting for several days, produces more marked effects. Plasma FFA concentrations increase progressively and attain a variable maximum in the range 1500 to 2500 μeq/l between the 3rd and 5th days of fasting (Annison, 1960; Reid and Hinks, 1962c; Patterson, 1963, 1964); plasma glucose concentrations decrease to a degree which is variable but inversely related to FFA concentrations (Reid and Hinks, 1962c),

and plasma ketone concentrations increase moderately to 5 to 10 mg % (Annison, 1960; Reid and Hinks, 1962c).

In severe undernourishment, caused by a reduction in nutrient intake, the increase in concentration of plasma ketones cannot originate from butyrate production in the rumen, as this must be markedly reduced, and it is now considered that the increased rate of ketogenesis in such situations is an inevitable consequence of an increased rate of gluconeogenesis (Krebs,1965, 1966). Under normal circumstances FFA are oxidized in the liver to acetyl-CoA which combines with oxaloacetate to form citrate, which in turn is metabolized through the tricarboxylic acid cycle. In situations of severe glucose deficiency, however, FFA are metabolized through other pathways which have been the subject of prolonged discussion and speculation, the history of which has been traced by Reid (1968).

The most recent hypothesis has been advanced by Krebs (1965, 1966), who has drawn attention to oxaloacetate as the intermediary metabolite common to the pathways involved in gluconeogenesis and ketogenesis. In severe carbohydrate insufficiency the activity of enzymes controlling gluconeogenesis is increased, leading to a decrease in the amount of oxaloacetate available to combine with acetyl-CoA, and a consequent reduction in the rate of the tricarboxylic acid cycle. The only other pathway available for energy production in such a situation is the oxidation in the liver of FFA to acetyl-CoA, which, in the absence or scarcity of oxaloacetate, leads to the formation of acetoacetate and subsequently β-hydroxybutyrate.

Approximately 75% of the ketones in the liver and blood of normal animals are present in the form of β-hydroxybutyrate, most of the remainder being acetoacetate plus small quantities of acetone (Reid, 1960). The ratio of β-hydroxybutyrate to acetoacetate in the liver does not vary appreciably from 3 : 1, even when the rate of ketogenesis increases markedly, as in starvation or severe undernourishment. In the blood, however, the ratio of β-hydroxybutyrate to acetoacetate increases to 6 : 1 to 11 : 1 with increasing ketone concentration (Reid, 1960; Berry, Williamson and Wilson, 1965). The rate of formation of ketones is determined by the equilibrium established between the rates of FFA oxidation and the entry of acetyl-CoA into the tricarboxylic acid cycle; the concentration of ketones in the blood is determined by the difference between the rates of production in the liver and utilization by extrahepatic tissues.

The concentration of FFA in plasma is determined by the rate of release of FFA from adipose tissue (Fritz, 1961). It has also been shown that the rate of FFA utilization is proportional to the plasma concentration (West and Annison, 1964), and thus this parameter constitutes a useful index of the rate of fat mobilization. The rate of release of FFA from stored fat, and consequently the plasma concentration and rate of utilization of FFA, are affected by a variety of nutritional and endocrine factors which have been the subject of a very large number of published reports and many comprehensive reviews (e.g. Jeanrenaud, 1961; Rudman, 1963; Knobil and Hotchkiss, 1964; Lands, 1965; Masoro, 1966; Olson, 1966; Shapiro, 1967). The great majority of reports on fat metabolism and factors affecting

plasma FFA concentrations have been concerned with human subjects or other monogastric species, and data on nutritional and endocrine effects in ruminants are limited. Mechanisms and pathways involved in fat metabolism in the ruminant have been reviewed by Tove (1965).

As already indicated, a reduction in energy intake to a sub-maintenance level, or the complete withdrawal of feed, results in an immediate increase in circulating concentrations of FFA in sheep (Annison, 1960; Reid and Hinks, 1962c; Patterson, 1963, 1964) and in cattle and goats (Hartmann and Lascelles, 1965; Menahan, Schultz and Hoekstra, 1966b; Radloff et al, 1966). Levels quickly return to normal on refeeding. In ruminants with elevated plasma FFA concentrations the provision or administration of readily utilizable energy in the form of acetate (Lindsay, 1959b), propionate (Patterson, 1963, 1964), glucose (Annison, 1960; Patterson, 1964), glycerol (Patterson, 1964) or β-hydroxybutyrate (Menahan et al, 1966b) decreases the rate of fat mobilization and lowers the concentration of plasma FFA.

Endocrine factors causing elevations in plasma FFA concentrations in ruminants include prolactin (Williams, Weisshaar and Lauterbach, 1966), adrenocorticotrophin (Radloff and Schultz, 1966), glucocorticords (Radloff and Schultz, 1966) and the catecholamines, adrenaline and noradrenaline (Lindsay, 1961; Radloff and Schultz, 1966). Insulin (Annison, 1960; Radloff and Schultz, 1966) and growth hormone (Williams, Lee, Head and Lynch, 1963; Manns and Boda, 1965; Bassett and Wallace, 1966; Radloff and Schultz, 1966) both cause transistory decreases in plasma FFA concentrations, followed by increases to levels higher than those pertaining before treatment.

In general, plasma FFA responses to hormone administration are in the same direction in ruminant and monogastric species, although the magnitude of the response in ruminants is frequently less than that in other animals and in man (Radloff and Schultz, 1966).

Plasma FFA concentrations are also increased, at least in humans and other monogastric species, by exercise (Basu, 1960; Basu, Passmore and Strong, 1960; Issekutz, Bortz, Miller and Wroldsen, 1967) and by exposure to cold (Masoro, 1966).

In normal circumstances, the two most important factors controlling FFA concentration are the availability and utilization rate of glucose. A change in either or both of these factors leads to an alteration in the lipogenesis-lipolysis equilibrium which is reflected in circulating FFA levels (Patterson, 1964). This inverse quantitative relationship between the two principal sources of energy is the basis of the "caloric homeostasis" described by Fredrickson and Gordon (1958).

It is pertinent at this stage to draw attention to factors affecting plasma FFA concentration which can limit the usefulness of this parameter in certain situations. The first concerns the considerable lability of FFA concentration and its susceptibility to adrenaline secretion following any form of emotional disturbance or stress. It is well established in humans that emotional factors cause the release of catecholamines into the circulation, and that this results in an almost immediate increase in the concentration of plasma FFA. Gottschalk, Stone, Glaser and Iacono (1966) have recently demonstrated a significant positive correlation in humans between anxiety levels during dreaming and changes in plasma FFA

concentration. Results of experiments with sheep (Patterson, 1963) suggest that potentially stressful situations, such as excessive handling and tentative venepunctures, may result in increased rates of fat mobilization and increased plasma FFA concentrations. It is therefore important in using plasma FFA concentration as an index of nutritional state that all possible precautions are taken to minimise emotional disturbance. This generally requires some element of training experimental animals to the procedures involved in blood sampling, and adherence to an established routine.

A second factor which must be considered in the interpretation of plasma FFA concentration is the apparent inability of at least ruminants to maintain very high FFA concentrations (in excess of, say, 2000 μeq/l) over prolonged periods. Menahan, Schultz and Hoekstra (1966a) noted that in fasted phloridzinized goats elevated plasma FFA concentrations were lowered when marked ketone body accumulation occurred, and attributed this to a feedback mechanism controlling ketogenesis from mobilized fat. A similar response has also been noted in sheep subjected to continued severe undernourishment (Reid, unpublished) and illustrates the inadvisability of using only a single parameter as an index of nutritional state.

It will be evident from these and earlier considerations that the severity of carbohydrate insufficiency or degree of undernourishment can be characterized by circulating concentrations of ketones and FFA. Although the primary stimulus to FFA mobilization and its attendant ketogenesis is a glucose insufficiency, plasma glucose concentrations do not necessarily constitute the most satisfactory index of undernourishment. As already indicated, minor changes in

plasma concentration may be associated with major changes in rate of utilization; endocrine factors may also affect either plasma concentration or utilization rate without affecting the other (Bassett, Mills and Reid, 1966). Nevertheless, plasma glucose concentration can be a useful parameter of nutritional state in a variety of situations, particularly when considered in relation to other parameters such as FFA or ketone concentrations.

Undernourishment occurs most commonly during pregnancy, particularly in hill sheep, and is a result of the high energy requirements of the foetus. The principal sources of energy in the foetus are glucose and fructose, the latter being synthesized in the placenta from maternal glucose. Reid (1968) has estimated, on the basis of calculations by Kronfeld (1958) and Bergman (1964), that the daily uptake of maternal glucose by the foetus is of the order of 8 to 9 g per kg foetal tissue. Lack of information regarding foetal weights, and the difficulties of comparing nutrient intakes in a variety of experiments make it difficult to estimate the proportion of maternal glucose supplied to foetal tissues, but it is evident from available information that the proportion is considerable. Work by Scarisbrick and Pugh (1957) indicates that acetate makes little contribution to foetal energy supplies, and studies by Reid (1962) have shown that only very small amounts of energy are likely to be derived from ketones, which in the foetus are present almost wholly in the form of acetoacetate. Little is known regarding the metabolism of FFA by the foetus. The high energy requirements of the foetus have been confirmed in calorimetric experiments by Graham (1964), who calculated the additional heat production due to foetal tissues to be

90 kcal per kg foetus per 24 hr. In reviewing foetal energy requirements, Reid (1968) concludes that "the hypoglycaemia so readily induced in pregnant ewes by undernourishment in late pregnancy is a consequence of high foetal demand for glucose from the limited supply available as a result of gluconeogenesis from propionate and amino acids. It also seems likely that the situation is aggravated, as far as the ewe is concerned, by a high efficiency of foetal glucose uptake".

The parameters considered in this section have been used on innumerable occasions to characterize the nutritional state of undernourished and adequately fed animals. They have frequently been used as the means of assessing effects of various nutritional treatments, and on occasions as the final criteria by which the effects of imposed treatments have been judged. Reid and Hinks (1962a), however, extended the use of these parameters in an experiment in which plasma ketone concentrations were used as a means of controlling nutritional state. Instead of feeding all animals in a particular treatment group the same predetermined amount of feed, and using plasma ketone concentrations to characterize the resulting nutritional states, Reid and Hinks adjusted individual feed intakes according to plasma ketone concentrations, thereby maintaining a constant nutritional state in all individuals. The use of this technique in an experiment with ewes during late pregnancy enabled these workers to present their results in terms of the feed required to maintain the predetermined nutritional state. This obviously varied with foetal weight, and is in contrast to the conventional experimental design in which changing requirements are illustrated,

but not measured, by the increasing severity of undernourishment as indicated by the principal biochemical parameters used.

The use and limitations of biochemical parameters as indices of, and means of controlling, nutritional state has been discussed by Reid and Hinks (1962a, 1962b).

III - OBJECTIVES OF EXPERIMENTAL WORK

It is difficult at this stage to present a detailed and precise outline of the experiments reported in a subsequent chapter. In some instances the objectives and design of a particular experiment were dependent on the results of earlier work, and cannot be properly considered without some knowledge of these results. In other instances more than one experiment was required to provide the information necessary to proceed to the next logical experiment. Selected data from experiments not included in this thesis have been used as evidence in support of various hypotheses suggested by earlier experiments; full details of such experiments are irrelevant in the context of this thesis, and the reasons for including selected data become clear only in the light of knowledge obtained from other experiments. The following chapter also contains examples of experiments which have been designed to provide information on two or more distinct but related aspects of a particular problem, and it has been considered expedient to examine these contributions separately. For these reasons it is convenient to consider the results of the experimental work under seven separate headings, some of which contain more than one experiment and some dealing with only part of a more complex experiment. A brief outline of the experimental work is given below under these headings.

(i) Changes in weight and body composition during pregnancy

The objective of this work was to provide information regarding the widely recognized, but poorly documented, changes in live weight and body composition of hill ewes during the pregnancy period.

(ii) The characterization of nutritional state during late pregnancy

The two investigations considered in this section constituted an attempt to characterize, in terms of the concentrations of certain blood metabolites, the nutritional state of free-grazing pregnant hill ewes kept under two traditional hill management systems.

(iii) Factors affecting the degree of undernourishment during late pregnancy

Results of the investigations referred to in section (ii) above led to the formulation of an hypothesis regarding the principal factors determining the nutritional state of individual ewes during late pregnancy. In this section evidence from one of the investigations in section (ii) and from three other unrelated experiments is presented in support of this hypothesis.

(iv) Metabolic responses to induced hypoglycaemia

The objective of this study was to examine the use and efficiency of different biochemical parameters as indices of undernourishment.

(v) The use of biochemical parameters in controlling nutritional state

This section deals with the experimental use of biochemical parameters as a means of maintaining prescribed nutritional states in individual ewes during late pregnancy, as opposed to the more usual and purely descriptive use of these parameters as indices of undernourishment.

(vi) The effect of undernourishment during pregnancy on lamb birth-weight

The experiment on the use of biochemical parameters in controlling nutritional state was also designed to provide data on two

other points of interest. This section deals with the effects of different levels of undernourishment during late pregnancy on lamb birth-weight.

(vii) Energy requirements of the pregnant ewe

The design of the experiment referred to in sections (v) and (vi) above also furnished data from which estimates were made of the energy requirements of pregnant ewes.

IV - MATERIAL AND METHODS

The aim, in this chapter, is to deal in a general manner with the material and methods used in experiments described in the following chapter. Details of certain techniques and analytical methods, some of which were used in a number of experiments, are also presented.

1. Location of Experiments

The research stations on which the majority of experiments were carried out belonged to the Hill Farming Research Organisation, and are described in the Organisation's first report (Hill Farming Research Organisation, 1958).

Investigations concerning the characterization of the nutritional situations existing under traditional management systems were conducted at Lephinmore, on the east side of Loch Fyne, Argyll. This farm, which rises from sea level to 1540 ft, has a high annual rainfall averaging about 70 in near sea level. The 2800 acres of hill grazing are mostly covered with peat, and have a mixed wet heath, blanket bog type vegetation.

Experiments requiring relatively large numbers of individually penned sheep were carried out at Glensaugh, which lies at the eastern end of the Grampians, in Kincardineshire. Sheep were kept in pens, each of approximately 16 sq ft, in the sheephouse and on an adjacent wooden slatted area outside.

Data are also presented from an experiment conducted on an enclosed area of improved pasture at Sourhope, on the western slopes of Cheviot in Roxburghshire.

Other investigations were carried out on sheep housed at Fulford Farm and in the sheephouse at Castlelaw, both belonging to The Edinburgh School of Agriculture, and part of their estate at Bush House, Midlothian.

2. Experimental Animals

Scottish Blackface sheep were used in most of the experiments, and, except where otherwise stated, were mature ewes having had at least two previous lamb crops (i.e. 3 years of age or older). At Lephinmore, Glensaugh and Castlelaw the ewes comprised part of the hill sheep stock of these farms. In the Sourhope experiment the sheep were mature South Country Cheviot ewes subsequently used in a lactation experiment carried out in collaboration with Eadie, and reported by Eadie (1967). Scottish Blackface ewes from Glensaugh were used in the experiment at Fulford.

3. Feeding

In the investigation conducted at Lephinmore concerning live-weight change and body composition the ewes were wholly dependent on available hill herbage for their nutrient supplies, and remained on the hill throughout the 12-month period of study. In the other investigation at Lephinmore, dealing with the characterization of nutritional state in late pregnancy, ewes received a daily supplement of approximately 100 g per head of a proprietary high protein concentrate, fed twice weekly for about five weeks, before being moved to sown pasture two weeks before the mean date of parturition. The supplementary feeding was continued during the period on pasture.

In the experiment at Sourhope the nutrient contribution from available grazing was negligible, and the ewes received a daily allowance of 1 kg medium quality hay plus approximately 100 g proprietary concentrate per head, fed on a group basis.

At Glensaugh the ewes in individual pens were fed a mixed diet of hay and a pelleted concentrate once daily, as described in the following chapter. It was estimated, from analyses carried out by the North of Scotland College of Agriculture, that the hay supplied 52 g digestible organic matter (DOM) per 100 g air-dry material. The concentrate (67% grass meal, 18% maize, 10% soya bean meal, and 5% molasses, plus added calcium, phosphorus, and vitamins A and D) supplied 66 g DOM per 100 g air-dry material, as determined both *in vitro*, by the method of Alexander and McGowan (1961) and *in vivo*, using three adult wether sheep.

This concentrate was also used as the diet in the Fulford experiment, when it was fed at a level of 15 g per kg live weight once daily.

Details of the nutrition of the sheep in the Castlelaw study are contained in the Hill Sheep Reports of the Edinburgh and East of Scotland College of Agriculture (1966).

4. Blood Sampling and Sample Preparation

The prime consideration in the procedure adopted for collecting blood samples was to minimize, and if possible avoid, any disturbance of the ewes which would be likely to cause emotional or psychological stress leading to even transient fat mobilization and elevated plasma FFA concentrations. Wherever possible necks were closely clipped

over each external jugular vein on a day samples were not being collected. During sampling sheep were held, with a minimum of restraint, in a normal standing position.

In the field studies at Lephinmore, previously identified ewes were collected for blood sampling, into small pens sited on the hill. Jugular venepunctures were made with 16 s.w.g. needles, using stasis, and 15 to 20 ml of blood collected directly into 1 oz glass bottles. Blood analysis indicated that after the first two occasions the ewes had become accustomed to the procedures involved and were not detectably disturbed. Evidence of disturbance was also looked for on a number of occasions throughout the periods of study, by collecting second samples from five or six ewes some 15 minutes after the withdrawal of the first sample. Plasma FFA concentrations in the second samples were never significantly higher than those in the earlier samples, and it was concluded that the procedure adopted was satisfactory.

In the more closely controlled experiments at Glensaugh and Fulford blood samples were collected from ewes in their individual pens or metabolism crates. These ewes were sampled relatively frequently and syringes fitted with 18 s.w.g. needles were used for the withdrawal of blood to eliminate the need for stasis and to lessen the risk of haematomata in the jugular region. Deliberate attempts were made, by neck clipping and simulated sampling, to accustom these animals to the procedures involved. Results of blood analyses again indicated that samples were collected without any detectable disturbance of the animals. In these and other experiments in which animals were fed once daily, all blood samples were collected before feeding.

Heparin was used as an anticoagulant in all samples at a concentration of 10 IU per ml of blood.

Heparinized blood samples were centrifuged, as soon after collection as possible, in 15 ml tubes at 2400 g for 15 to 20 minutes. Plasma was removed with a syringe after centrifugation and, if not required immediately for analytical purposes, stored in stoppered polystyrene tubes at -20°C.

5. Analytical Methods

(a) Body composition

As one of the objectives of the body composition study was an investigation of changes in the distribution of fat throughout the tissues of the ewe, it was necessary to separate the various organs and parts at an early stage in the procedure. The ewes were shorn immediately prior to slaughter at Lephinmore. After slaughter, freely draining blood was collected and weighed, and the internal organs plus the omental and mesenteric fat depots were removed and weighed individually. Gastrointestinal contents were estimated by weight difference. Carcasses were dressed according to commercial practice to satisfy the requirements of an allied investigation (Russel, Gunn, Skedd and Doney, 1968) and were stored together with the other tissues for five to six months at -20°C.

Before thawing, the carcasses were divided down the back-bone with a meat band-saw and both halves weighed. Left sides of the carcasses were dissected into four components: subcutaneous fatty tissue, perirenal fatty tissue, muscular tissue plus associated fatty tissue (i.e. both intermuscular and intramuscular fat), and

bone. These components and the pericardial fatty tissue, omental plus mesenteric fatty tissues, liver, and foetal tissues (where present) were individually weighed, minced and homogenized prior to sampling for chemical analysis. The remaining parts and organs (including head, skin, kidneys, viscera, metacarpals and metatarsals, but excluding horns, hooves and fleece) were minced and homogenized together, and thereafter referred to collectively as the "remainder".

Accurately weighed, homogenized, triplicate samples (each of approximately 10 g) of minced subcutaneous, perirenal, pericardial, and omental plus mesenteric fatty tissues, muscular tissue plus associated fatty tissue, bone, liver, and remainder were dried to constant weight for approximately 40 hr in aluminium foil cups at 105°C. Moisture contents were calculated by difference. The dried material was crushed in the aluminium cups which were then perforated and folded, and, without further weighing, Soxhlet-extracted with petroleum ether (60-80°C) for 8 hr. Fat contents were estimated by weight differences of Soxhlet flasks after evaporation of the petroleum ether.

In calculating body and tissue compositions in terms of moisture, fat (i.e. ether-extractable material) and fat-free dry matter, weight losses between slaughter and preparation for chemical analysis were considered to be 100% water. Blood collected at slaughter was not analysed, but was assumed to contain 79% water and negligible amounts of fat (White, Handler and Smith, 1964).

The weight of the 'maternal empty body' (MEB) was computed as the sum of all maternal tissues and consequently excluded certain components of live weight, viz. the fleece and the contents of the

gastrointestinal tract and gravid uterus. In computing the weight of certain MEB components it was necessary to make adjustments for inevitable small differences in weight (less than 1%) between the two halves of each carcass. Although these differences undoubtedly affected composition, it was considered that the effect was sufficiently small to be ignored. These corrections were applied to the four carcass components of MEB weight. The non-carcass components of MEB weight were pericardial and omental plus mesenteric fatty tissues, liver, remainder and allowances for water lost in shrinkage and in blood.

(b) Plasma glucose

Plasma glucose concentrations were determined in the earlier experiments by the manual method of Huggett and Nixon (1957) and in later experiments by an automated method using a Technicon Auto-Analyser. Both methods are highly specific for glucose, being based on the use of glucose oxidase to convert glucose to gluconic acid plus hydrogen peroxide. The peroxide is subsequently reduced by peroxidase, and the released oxygen reacts with a chromogen to produce a colour which is then measured photometrically.

In the manual method, which was carried out on protein-free plasma filtrates prepared by the method described by Somogyi (1952), it was found that the quantity of glucose oxidase could be reduced to 12% of that quoted by Huggett and Nixon (1957) without loss of sensitivity.

The automated method was a modification of the Technicon glucose oxidase-peroxidase technique, and was adopted in preference to the more commonly used automated method which estimates total reducing

sugars and is not specific for glucose. Techniques of this type, which are based on enzyme reactions, are frequently sensitive to small variations in temperature and time, and are thus well suited to automation where both these variables can be closely controlled. The automated method has an additional advantage in that it incorporates a dialyser which eliminates the need for protein precipitation.

(c) Plasma ketones

Concentrations of plasma ketone bodies were determined as mg acetone per 100 ml plasma by the single distillation method of Reid (1960), using protein-free plasma filtrates prepared, as in the manual glucose method by the method of Somogyi (1952). This method is based on the conversion of all ketone bodies to acetone, which is distilled and subsequently determined colorimetrically following its reaction with ethanolic salicylicaldehyde to form dihydroxydibenzene acetone in alkaline solution.

In this method the recovery of acetone from acetoacetic acid is virtually 100%, and that from β-hydroxybutyric acid of the order of 70 to 75%. The single distillation method involves the assumption of a constant ratio of acetoacetic acid to β-hydroxybutyric acid, and any deviation from this ratio, as may be expected in hyperketonaemic ewes (see previous chapter), will lead to inevitable errors in the estimation of plasma ketone concentrations. Reid (1960) has calculated, however, that these errors must be small in relation to circulating concentrations of ketone bodies.

(d) Plasma free fatty acids

Plasma FFA concentrations were determined by the method described by Patterson (1963) in which FFA are extracted by a slightly acidified

mixture of isopropanol and heptane. In this method the heptane fraction containing the extracted FFA is evaporated to dryness to remove the lower steam-volatile fatty acids and ketone bodies. The FFA are then redissolved in warm ethanol and titrated against weak alkali, using a Conway micro-burette, with an ethanolic Nile blue solution as indicator. It was found that the end point of the titration was improved by adding chloroform to the ethanol (2 : 5 v/v) used to redissolve the FFA. Incorporation of the indicator in the chloroform: ethanol system, as opposed to its addition at each titration, improved the precision of the technique.

6. Statistical Methods

Correlation coefficients, regression equations, analyses of variance and covariance, and tests of significance were all computed according to standard statistical techniques as described by Snedecor (1957).

V - RESULTS

1. Changes in Weight and Body Composition During Pregnancy

The objectives of this investigation were, firstly, to study changes in live weight and in the weight of maternal tissues of free-grazing pregnant hill ewes between mating in November and parturition in April, and, secondly, to examine the components of these changes with particular regard to the distribution of body fat. Although attention was focused on the pregnancy period, a subsidiary objective was the establishment of the pattern of live-weight change throughout the annual cycle.

One hundred and eighty Scottish Blackface ewes of mixed ages grazing on hill pasture at Lephinmore were weighed at approximately fortnightly intervals during the year October 1964 to September 1965. Twenty-four of these ewes, selected at random from the five-year-old age group, were used for body composition studies. Six were slaughtered in early November before mating, a further six in March at about the 16th week of pregnancy, and 11 during the last week of pregnancy in April. One ewe which lambed shortly before it was due to be slaughtered was discarded from the study. Two of the ewes slaughtered in March and four of those slaughtered in April were carrying twin foetuses. None of the ewes was barren. No significant differences were found between single- and twin-bearing ewes in any of the variables measured, and the effect of number of foetuses is not considered further. All ewes were in approximately the same subjectively assessed body condition at the beginning of the experiment, equivalent to grade 2 to 2.5 on the scale given by Jefferies (1961) which ranges from 0 (extremely emaciated) to 5 (very fat).

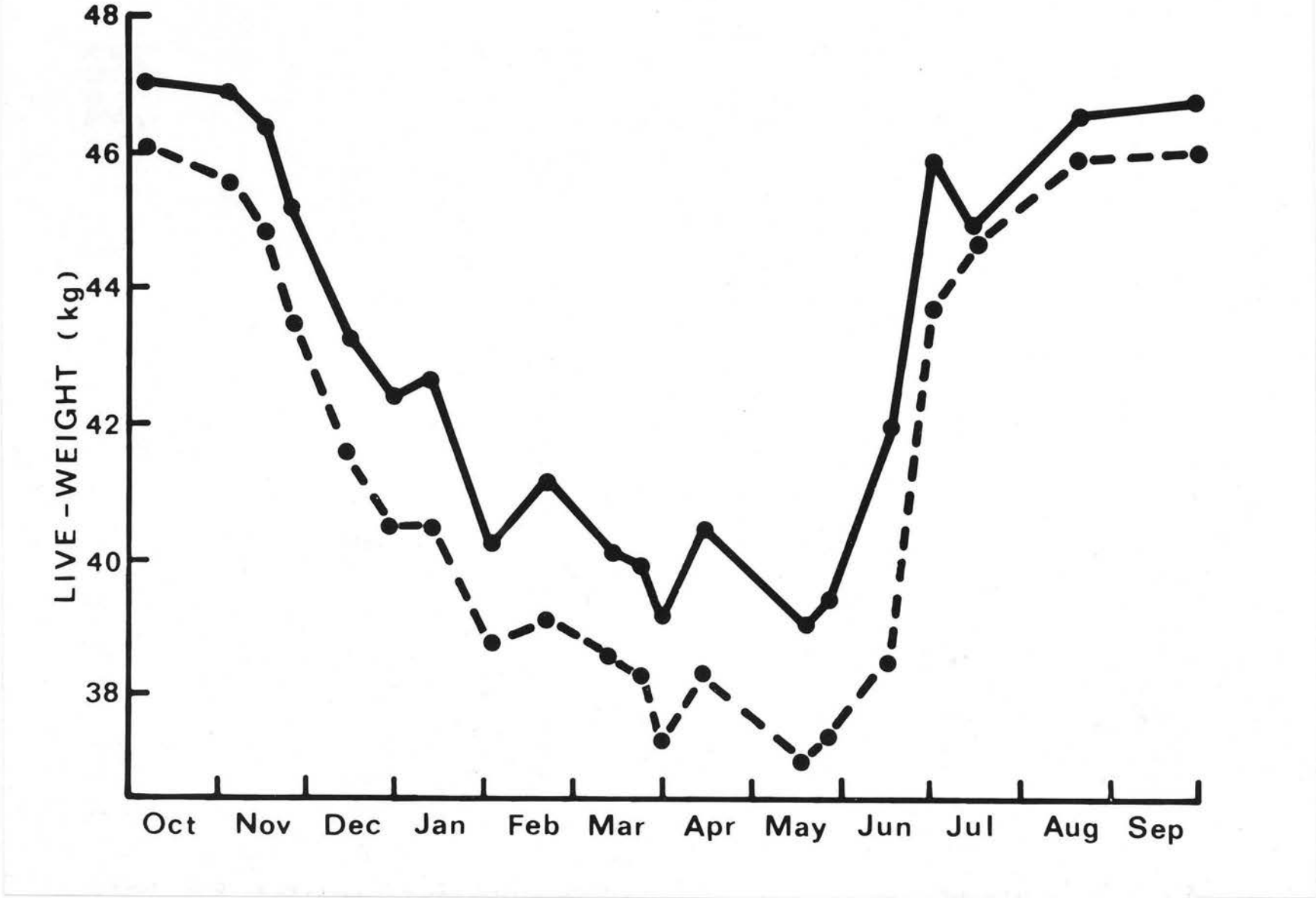

Fig. 3. Annual changes in mean ewe live weight. The broken line represents mean live weight corrected for fleece growth and moisture retained by the fleece.

Mean live weights throughout the year of the 180 ewes comprising the hill flock are presented in Figure 3. Live weight was at a maximum for a short period in late autumn, and declined progressively from before mating in late November until about the time of parturition. During and after lactation live weight increased until the maximum was again reached in late autumn. The amplitude of the mean live-weight curve was approximately 8 kg. For reasons which will be discussed later, it is likely that this figure underestimates the live-weight change of individual animals.

Data relating to the 23 ewes slaughtered for composition studies were adjusted by covariance analysis for between-group differences in initial live weight. Adjusted means of live and maternal empty body (MEB) weights and weights of MEB components are presented in Table 3. (The original data are contained in Appendices 1 - 3). There were significant and approximately parallel decreases in live and MEB weights between early November (i.e. when live weight was near maximum and some three weeks before mating) and mid-March (i.e. about the 16th week of pregnancy). During the final month of pregnancy there was a small but non-significant increase in live weight and a continued and significant decrease in MEB weight. At the first two slaughter dates there was a difference of 12 kg between live and MEB weights. This apparently constant component of live weight comprised approximately 11 kg gastrointestinal contents and 1 kg fleece in November, and 7.5 kg gastrointestinal contents, 3 kg uterine contents, and 1.5 kg fleece in March. At the end of pregnancy the difference between live and MEB weights had increased to 15.5 kg, and was made up of approximately 6.5 kg gastrointestinal contents, 7.5 kg uterine contents, and 1.5 kg fleece.

TABLE 3

Mean live and MEB weights and weights of MEB components (kg)

(Figures in parentheses are percentages of total MEB weight or weight change)

Slaughter group and date	Live wt at slaughter	MEB wt	Wt fat in MEB	Wt water in MEB	Wt fat-free DM in MEB
I 4.11.64	46.17	33.94	6.14 (18.1)	20.66 (60.9)	7.14 (21.0)
II 10.3.65	40.93	28.71	4.54 (15.8)	18.41 (64.1)	5.76 (20.1)
III 14.-21.4.65	42.05	26.53	3.00 (11.3)	17.81 (67.1)	5.72 (21.6)
Between group differences					
I - II	5.24***	5.23***	1.60 (30.6)	2.25** (43.0)	1.38 (26.4)
II - III	-1.12	2.18*	1.54* (70.7)	0.60 (27.5)	0.04 (1.8)
I - III	4.12**	7.41***	3.14*** (42.4)	2.85*** (38.5)	1.42 (19.1)

*P < 0.05 **P < 0.01 ***P < 0.001

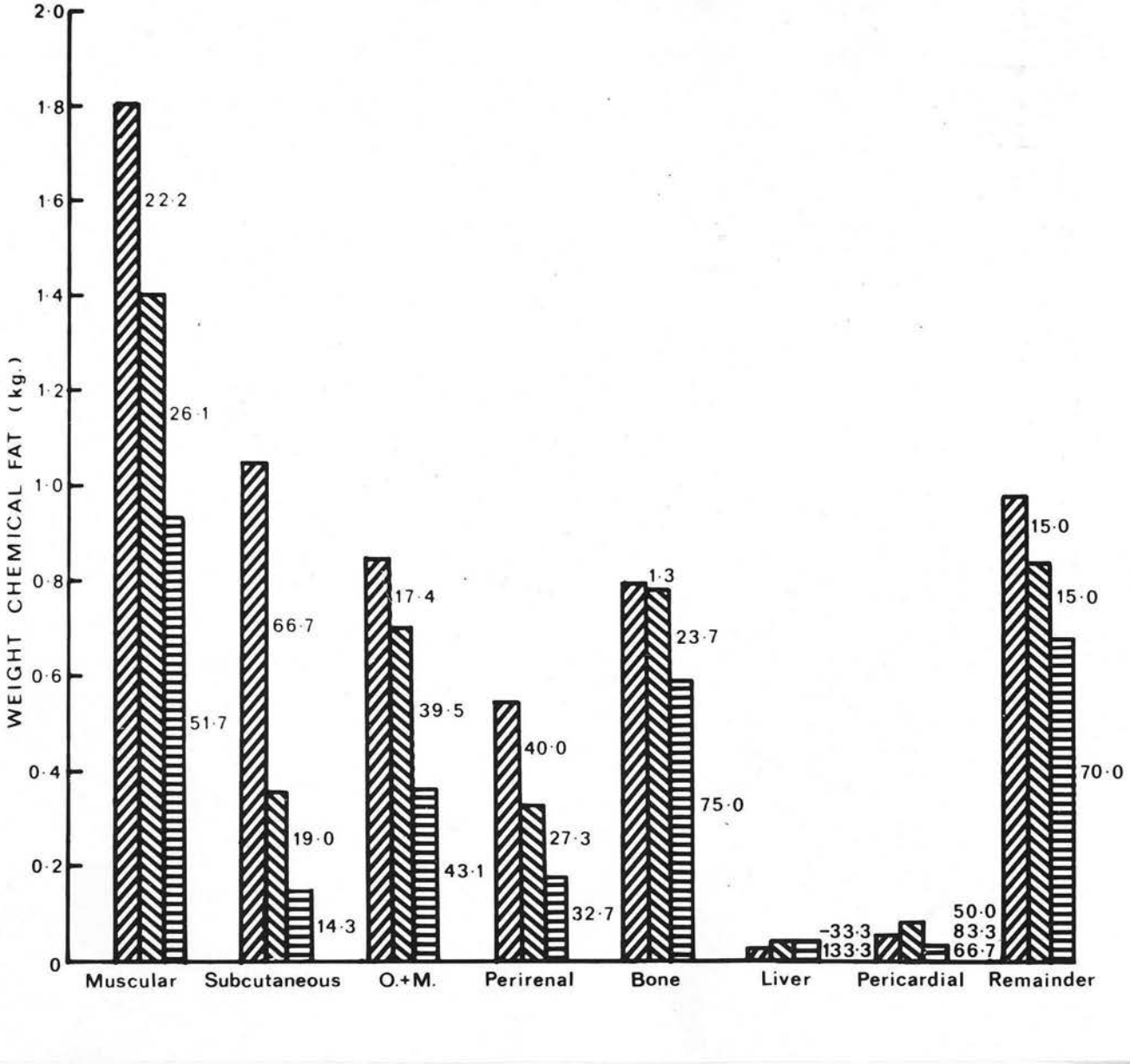

Fig. 4. Weight of chemical fat in eight depots in the maternal empty body before mating (1st column), at approximately 16 weeks pregnant (2nd column), and one week before parturition (3rd column). The figures refer to the percentage of fat lost from each depot between the first and second stages, between the second and third stages, and remaining at the third stage.

The weight of maternal tissue lost between the first two slaughter dates was more than twice that lost during the subsequent month; the rate of loss, however, was considerably greater during the second period. The composition of weight losses during these periods indicates that there was a considerable and highly significant water loss during the earlier stages of pregnancy, and that the rate of fat mobilization increased as pregnancy advanced. In the first four months the loss from maternal tissues (approximately 1.3 kg per month) contained more than 40% water and 30% fat, whereas in the final month the 2.2 kg lost contained less than 30% water and more than 70% fat (Table 3). The losses of what has been termed the fat-free dry matter, which consists mainly of protein and ash, were not statistically significant, but were in the same direction as, and in approximate proportion to, the losses of water. Water and protein are the principal constituents of muscular tissue and thus approximately parallel losses of these components are to be expected (Ulyatt and Barton, 1963).

The composition of the 7.4 kg weight loss (i.e. more than 20%) from maternal tissues during the pregnancy period represented 51% of the fat, 14% of the water and 20% of the protein plus ash present in the maternal tissues at maximum live weight.

Data relating to the distribution of fat throughout the maternal tissues at different stages of pregnancy are presented in Table 4 and Figure 4. Fat associated with muscular tissue constituted the largest single fat depot at the time of maximum live weight. The other principal sites of fat deposition, in decreasing order of magnitude, were: subcutaneous tissue, remainder, omental plus

TABLE 4

Mean weights of MEB fat components (kg)

(Figures in parentheses are percentages of total MEB fat weight or weight change)

Slaughter group	Wt. fat in MEB	Components of MEB fat		
		Muscular	Subcutaneous	Omental and Mesenteric
I	6.14	1.80 (29.3)	1.05 (17.1)	0.86 (14.0)
II	4.54	1.40 (30.7)	0.35 (7.7)	0.71 (15.6)
III	3.00	0.93 (31.0)	0.15 (5.0)	0.37 (12.4)
		Between group differences		
I - II	1.60	0.40 (25.0)	0.70*** (43.8)	0.15 (9.4)
II - III	1.54*	0.47* (30.5)	0.20 (13.0)	0.34* (22.1)
I - III	3.14***	0.87*** (27.7)	0.90*** (28.7)	0.49** (15.6)

TABLE 4

Mean weights of MEB fat components (kg)

(Figures in parentheses are percentages of total MEB fat weight or weight change)

Components of MEB fat				
Perirenal	Bone	Liver	Pericardial	Remainder
0.55 (9.0)	0.80 (13.0)	0.03 (0.5)	0.06 (1.0)	0.99 (16.1)
0.33 (7.2)	0.79 (17.4)	0.03 (0.6)	0.09 (2.0)	0.84 (18.8)
0.18 (6.0)	0.60 (20.0)	0.04 (1.3)	0.04 (1.3)	0.69 (23.0)
Between group differences				
0.22 (13.7)	0.01 (0.6)		-0.03 (-1.9)	0.15 (9.4)
0.15 (9.7)	0.19* (12.3)	-0.01 (-0.6)	0.05* (3.3)	0.15 (9.7)
0.37*** (11.8)	0.20** (6.4)	-0.01 (-0.3)	0.02 (0.6)	0.30** (9.5)

mesenteric fatty tissues, bone, and perirenal fatty tissue. Fat in the liver and pericardial fatty tissue, which collectively form only 1.5% of total MEB fat at maximum live weight, do not constitute significant fat reserves and are not considered further.

The principal points of interest in the data on fat distribution are centred on the different patterns of fat mobilization from individual tissues. Despite the statistically non-significant reduction in total weight of fat in the maternal tissues during the first four months of pregnancy (Table 3), there was a very highly significant decrease of 0.7 kg in the weight of subcutaneous fat (Table 4), representing 67% of the original subcutaneous reserves (Figure 4). This particular depot was depleted by a further 19% during the final month of pregnancy, making a total reduction in subcutaneous fat of 86%, and an overall contribution of 29% to the total weight of fat lost during the period of study. A very different pattern was evident in the mobilization of fat from skeletal tissue. During the first period only 1% of bone fat was withdrawn, but in the final stages of pregnancy there was a further reduction of 24% in the weight of bone fat, representing 12% of all fat lost during this period.

It is evident from the results of this investigation that the body tissues, and particularly the fat reserves, of free-grazing hill ewes are progressively depleted as pregnancy advances. The catabolism of substantial amounts of body tissues indicates clearly that the nutritional intakes of ewes at this time are insufficient to meet their requirements, and that these ewes must be undernourished. Results presented in the following section characterize, in physiological terms, the severity of the undernourishment implicit in the above data.

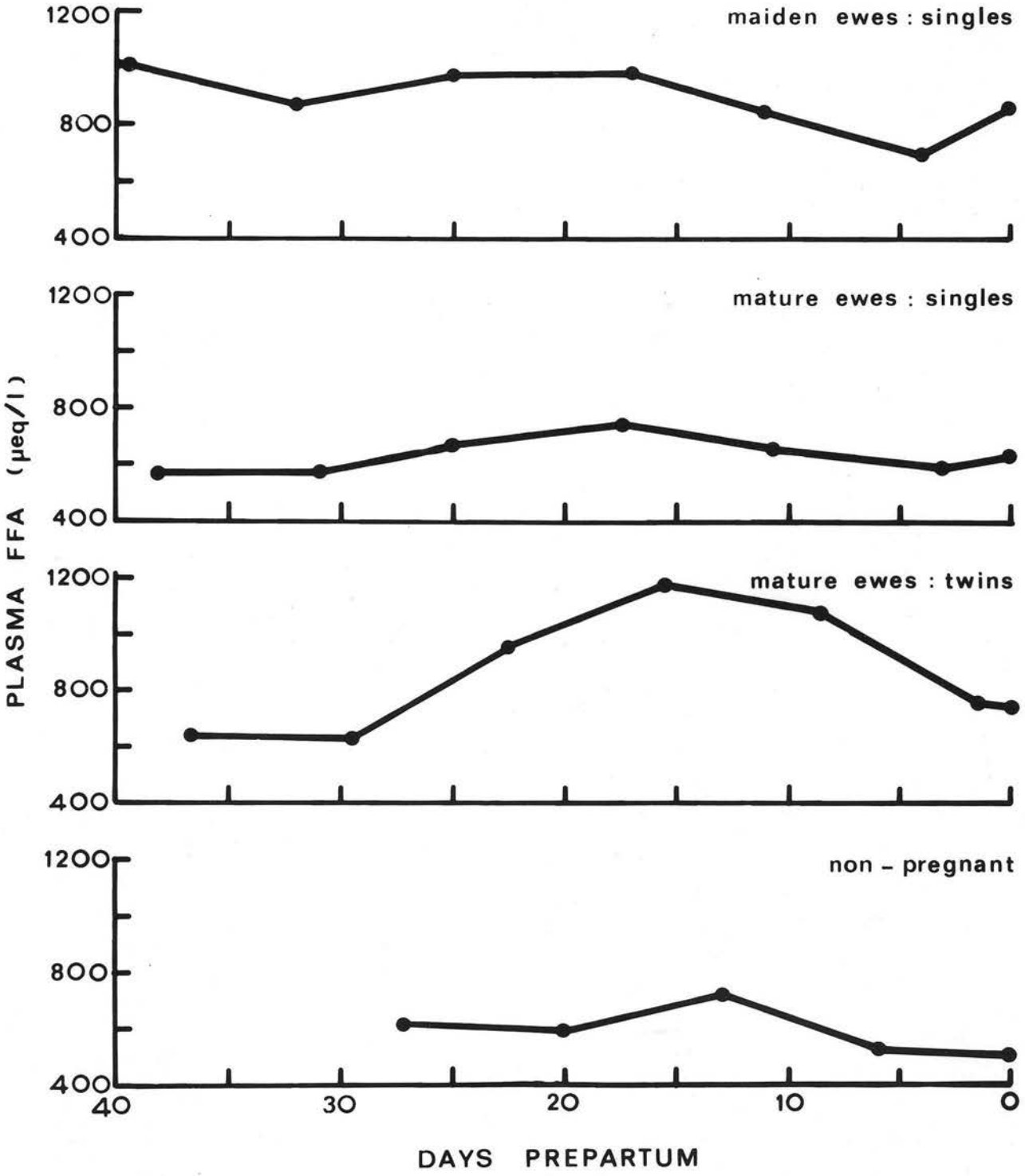

Fig. 5. Mean plasma FFA concentrations in four classifications of free-grazing hill ewes.

2. The Characterization of Nutritional State During Late Pregnancy

The common objective of two investigations considered in this section was to characterize, in physiological terms, the nutritional state during late pregnancy of ewes maintained under traditional hill management systems.

In the first study 21 mixed-aged Scottish Blackface ewes in a hill flock at Lephinmore were blood sampled weekly during late pregnancy. The management of the ewes, which received some supplementary feeding as described earlier, was considered to be typical of that in many Scottish hill flocks. All ewes were moved from the hill to sown pasture on the same date, although lambing was spread over a period of some three weeks. For this reason, and also because the time of lambing coincided with the initiation of grass growth, the earlier lambing ewes were at a disadvantage compared with those which lambed later and had a longer period of superior nutrition before parturition. Thus the mean plasma FFA concentrations, which are illustrated in Figure 5 and refer to four classifications of ewes, can be considered in only very general terms. (FFA concentrations in individual ewes are given in Appendix 4).

The mean plasma FFA concentration of 11 single-bearing mature ewes (i.e. ewes in their second or later pregnancy) increased from less than 600 μeq/l to 750 μeq/l during the fifth to third weeks prepartum, before declining as a result of the increased level of nutrition. A relatively more severe degree of undernourishment was noted in the two twin-bearing mature ewes, in which plasma FFA concentrations increased rapidly from 600 μeq/l four weeks before

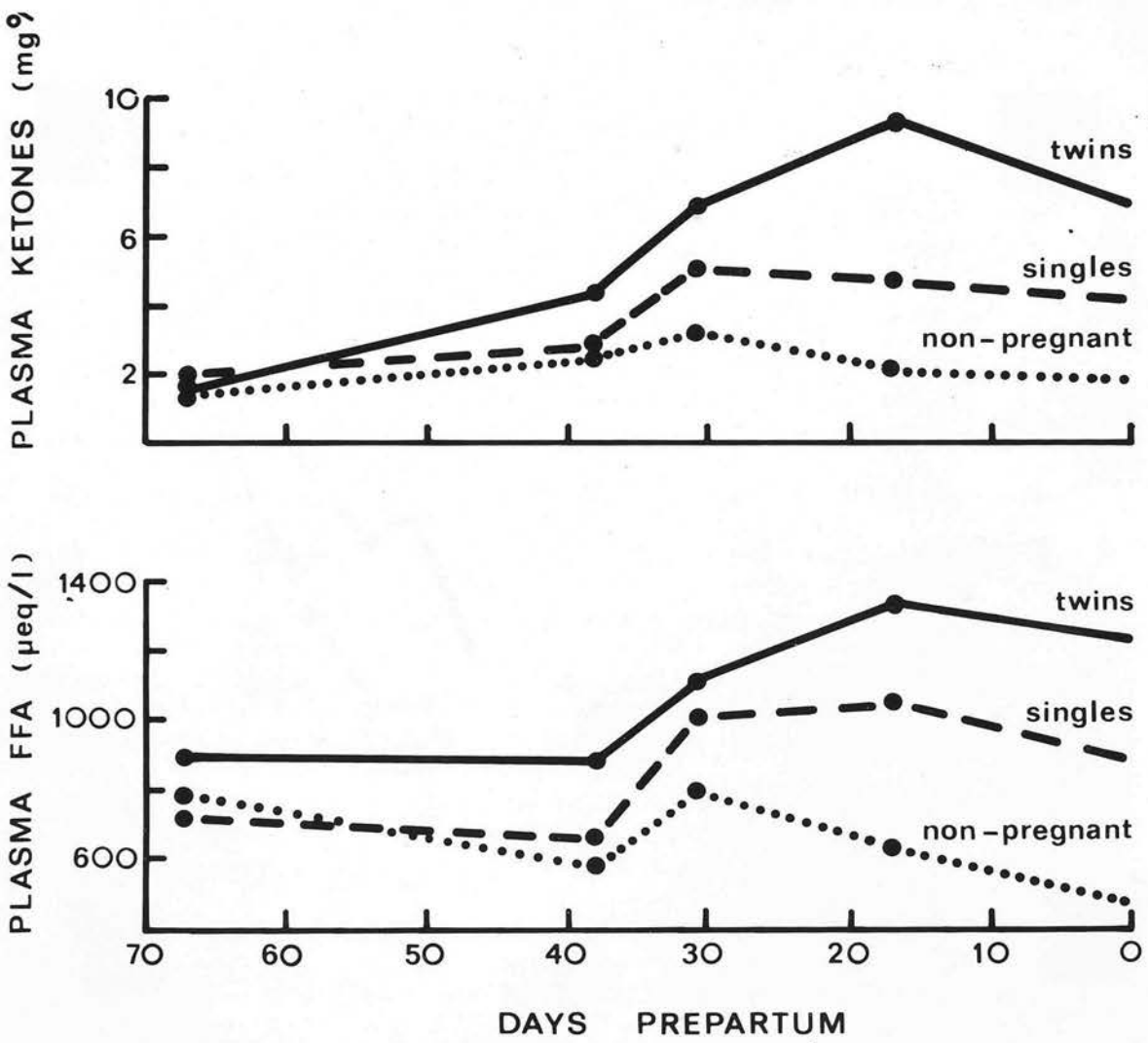

Fig. 6. Mean plasma ketone and FFA concentrations in three classifications of free-grazing hill ewes. The final sections of each line represent extrapolations to post-partum values (for explanation see text.)

parturition to almost 1200 μeq/l at two weeks prepartum. In five ewes in their first pregnancy, each with a single foetus, the mean plasma FFA concentration of approximately 1000 μeq/l indicated a degree of undernourishment which was almost as great as that in older ewes with twins, and was sustained for a longer period. The beneficial effect of moving the ewes to better pasture before parturition was reflected in the decreased FFA concentrations of all pregnant ewes, and in a further lowering of the already low FFA concentrations in three non-pregnant ewes.

In the second study 20 Scottish Blackface ewes in another hill flock at Lephinmore were blood sampled less frequently over a longer period. These ewes received no supplementary feeding at any time and remained on the hill throughout pregnancy. Mean plasma ketone and FFA concentrations of 5 twin-bearing, 13 single-bearing, and 2 non-pregnant ewes are illustrated in Figure 6. (These data are contained in Appendix 5). The apparently decreasing ketone and FFA concentrations during the last two to three weeks of pregnancy are extrapolations to post-parturient values; it is likely that the concentrations of these parameters continued to increase until parturition, and declined during the early post-parturient period. These ewes received no supplementary feeding, but the declining ketone and FFA concentrations in the non-pregnant ewes indicate an increase in the level of nutrient intake during the month before parturition, presumably as a result of grass growth at about this time. Although later lambing ewes would again have a nutritional advantage over those lambing earlier, this would be small in relation to that in the first study. It is also unlikely that this

presumed increase in nutrient intake would be sufficient to improve the nutritional status of the pregnant ewes, although it would undoubtedly help to slow the increasing severity of undernourishment.

The biochemical indices of undernourishment (Figure 6) again indicate that twin-bearing ewes were more severely undernourished than those with single foetuses. As might be expected, a comparison of plasma FFA concentrations with those obtained in the previous study (Figure 5) indicates that the ewes which were wholly dependent on hill grazing for their nutrient supply were more severely undernourished than those which received supplementary feeding and were moved to sown pasture before parturition. Mean plasma FFA concentrations in unsupplemented single-bearing ewes were of the same order as those in supplemented twin-bearing ewes. FFA concentrations in unsupplemented ewes with twin foetuses exceeded 1300 μeq/l and may have reached even higher levels immediately before parturition. In the unsupplemented ewes the maximum mean plasma ketone concentrations were 5.0 mg % in single-bearing ewes and 9.3 mg % in twin-bearing ewes, compared with 3.1 mg % in non-pregnant animals.

The results of these studies characterize the nutritional states of free-grazing pregnant hill ewes under two systems of management. In very general terms, single-bearing mature ewes may be described as moderately undernourished during late pregnancy, and single-bearing maiden ewes and twin-bearing mature ewes as severely undernourished. Measurable undernourishment was also observed in non-pregnant ewes, indicating that the nutrients available from hill grazings during late pregnancy were scarcely sufficient to meet maternal maintenance requirements.

It is also evident from the results that the practices of supplementary feeding and moving the ewes to intensively managed pasture before parturition alleviated the severity of undernourishment at this time.

3. Factors Affecting the Degree of Undernourishment During Late Pregnancy

Two further points of interest are evident in the results presented in the previous section. The first concerns similarities in the results of the two investigations, and the second arises from differences between the two sets of data. In both investigations twin-bearing ewes were more severely undernourished than those with single foetuses; ewes with single foetuses were, in turn, more severely undernourished than non-pregnant ewes. These observations suggest the possibility of a relationship between the severity of undernourishment and foetal weight. In general, ewes in the first investigation (i.e. those which received some supplementary feeding and were grazing intensively managed pasture before parturition) were less severely undernourished than those which were wholly dependent on hill herbage for their nutrient supply. Although no data on intake were available, it can be assumed with some degree of confidence that the level of nutrient intake was higher under the first management system, and that this higher level of intake was responsible for the superior nutritional status of these ewes.

These considerations lead logically to the formulation of the hypothesis that the degree of undernourishment in ewes during late pregnancy is determined by two main factors, viz. foetal weight and level of feed intake.

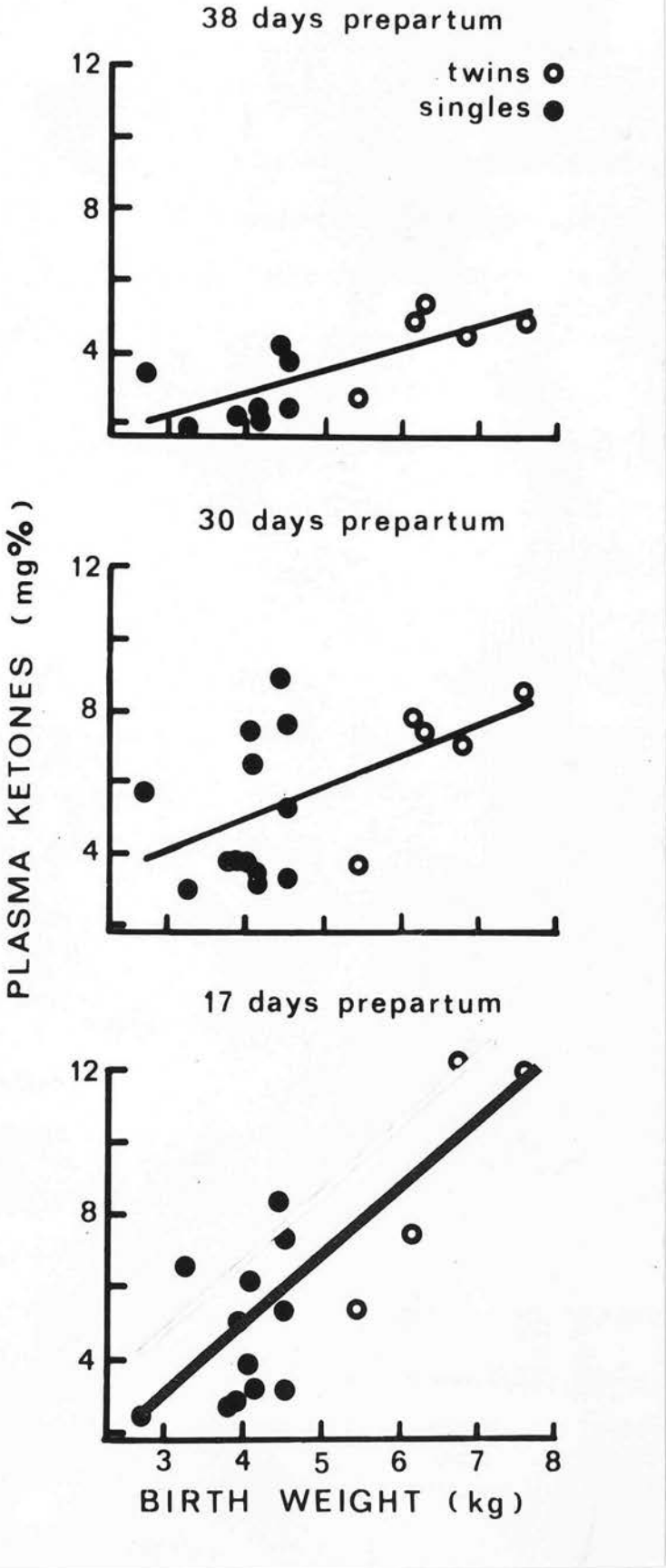

Fig 7. Relationships between plasma ketone concentrations at three dates before parturition and lamb birth-weights.

The conditions under which the field studies were carried out (i.e. conditions dictated by the stated objective) provided results from which the above hypothesis could be formulated, but did not fully meet the requirements necessary to obtain evidence on which the hypothesis could be conclusively confirmed or rejected. For example, in the first investigation the substantial increase in level of nutrition, associated with the change from hill to sown pasture, occurred at different time intervals from parturition in different individuals. This is likely to operate against the establishment of relationships between degree of undernourishment and foetal weight, as valid relationships must be based on data obtained at comparable stages of pregnancy from individuals on at least similar intakes.

In the second study lambing was also spread over a period of approximately three weeks, but nutritional changes under this management system were considered to be relatively small compared to those in the first investigation. In this second study statistically significant relationships were established between lamb birth-weights and plasma ketone concentrations on the three sampling dates before parturition, and between birth-weights and plasma FFA concentrations at the first and third prepartum samplings. (Birth-weights are included with details of the plasma parameters in Appendix 5). The relationships between plasma ketone concentration (mg %) (y) and birth-weight (kg) (x), which are illustrated in Figure 7 and presented below, attained higher statistical significance than the corresponding FFA relationships.

38 days prepartum	$y = 0.63x + 0.25$	$r = 0.76$
31 days prepartum	$y = 0.88x + 1.42$	$r = 0.53$
17 days prepartum	$y = 1.91x - 1.04$	$r = 0.80$

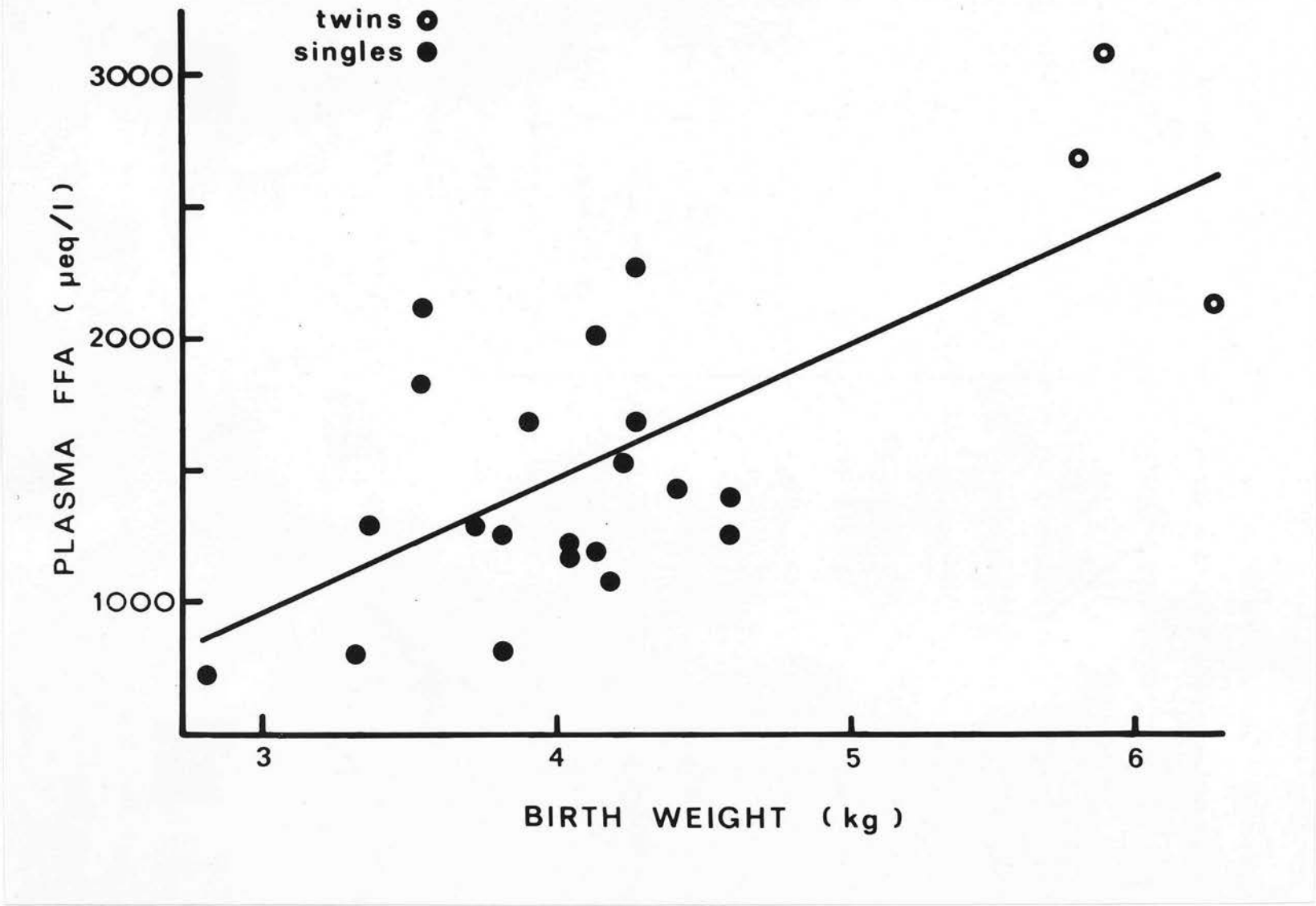

Fig. 8. The relationship between lamb birth-weights and plasma FFA concentrations during the final week of pregnancy in group-fed ewes with assumed intakes of 10–12 g DOM per kg live weight.

Although the number of ewes sampled varied slightly between sampling dates, as a result of occasional animals being missed in hill gatherings during unfavourable weather conditions, these relationships demonstrate that the degree of undernourishment (as measured by plasma ketone concentrations) in individual animals of this particular group was dependent on and significantly related to foetal weight. The increasing severity of undernourishment with advancing pregnancy, illustrated in Figure 6, is also evident in the changing values of the regression coefficient with time.

Further evidence of the dependence of degree of undernourishment on foetal weight comes from an experiment carried out at Sourhope in collaboration with Eadie. Although the experiment itself is not relevant in the present context, certain data from this work may be used to support the hypothesis outlined above. Plasma FFA concentrations were determined on blood samples collected from 23 South Country Cheviot ewes during the final week of pregnancy. All ewes lambed within a period of four days. The mean daily intake of these ewes, which were fed as a group, was of the order of 10 to 12 g DOM per kg live weight. A highly significant relationship was established between plasma FFA concentration (μeq/l) (y) and birth weight (kg) (x). (Data are given in Appendix 6). The regression equation, illustrated in Figure 8, was:

$$y = 508x - 576 \qquad r = 0.70$$

The undernourishment measured in these ewes was more severe than that encountered in either of the groups at Lephinmore, but the data show the same general pattern and provide additional evidence of the dependence of degree of undernourishment on foetal weight.

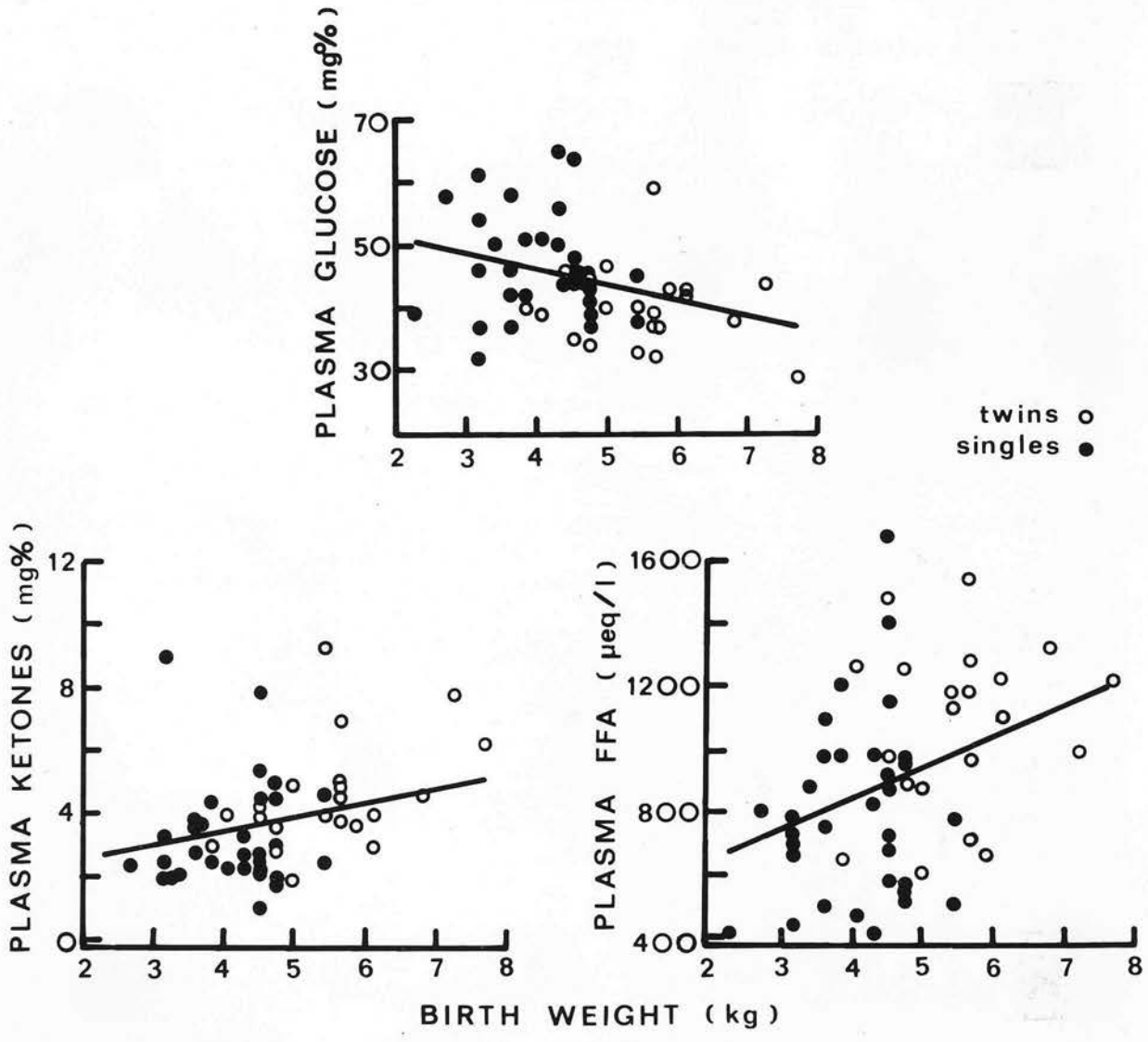

Fig. 9. Relationships between lamb birth-weights and plasma glucose, ketone and FFA concentrations in group-fed ewes three weeks before parturition.

In-wintering trials, conducted by The Edinburgh School of Agriculture, provided opportunities to characterize the nutritional state of pregnant ewes kept under a different type of management system, and to examine relationships between nutritional state and foetal weight in another situation. Blood samples were collected from two groups, each of 54 Scottish Blackface ewes, housed in a semi-open building. Levels of nutrient intake of the two groups were comparable, but ewes in Group B were treated with long-acting antibiotics five weeks before lambing. The samples, collected three weeks before lambing, were analysed for plasma glucose (mg %) (y_1), ketones (mg %) (y_2) and FFA (μeq/l) (y_3). In Group B all three plasma parameters were significantly related to lamb birth-weights (kg) (x). The relationships derived from data in Appendix 7, are illustrated in Figure 9 and presented below:

$$y_1 = -2.49x + 56 \quad r = -0.35$$
$$y_2 = 0.44x + 1.6 \quad r = 0.30$$
$$y_3 = 97x + 455 \quad r = 0.36$$

No significant relationships were found in Group A, the group which did not receive the antibiotic treatment, and in which a number of deaths due to a Pasteurella infection occurred. It is possible that an appreciable number of the ewes in this group were harbouring a subclinical infection at the time of sampling, and that this affected the results. The infection itself would be likely to increase the severity of the undernourishment, and could probably have reduced the ability of infected ewes to compete for nutrients with uninfected individuals under the group feeding system practised. Any increase in the variation in intake between individuals would

operate against the establishment of relationships between nutritional state and foetal weight. This theory of subclinical infection is highly speculative and cannot be proved, but it is supported by the fact that ewes in Group A were more severely undernourished, as indicated by all three plasma parameters, yet produced smaller lambs than ewes in Group B.

The results of all three studies considered so far in this section support the hypothesis regarding degree of undernourishment and foetal weight. The original hypothesis, however, included intake as a factor determining nutritional state, and although a certain amount of information was available in some of the studies regarding the intakes of different groups of ewes, individual intakes were not known. In all three situations there has been the assumption, implicit if not stated, that all ewes within a group had comparable intakes. If this assumption is valid then differences in degree of undernourishment between individuals are a reflection of differences in energy requirements, those ewes with the heaviest foetuses having the highest requirements. There is, however, no evidence for the validity of the assumption of equal intakes in all ewes within a group and it is possible that the significant relationships presented above could have arisen from differences in intake. It has been suggested (Reid, 1958), although the suggestion was later refuted (Reid and Hinks, 1962a), that there may be a negative relationship between voluntary intake and foetal weight or size. This would mean, of course, that ewes with heavier foetuses were more severely undernourished because of their lower intakes and not as a result of higher requirements.

The fourth and final set of data presented in this section deal with the relationship between degree of undernourishment and foetal weight in ewes eating equal amounts, per kg live weight, of a standard diet. A group of 16 Scottish Blackface ewes, kept in individual pens in the sheephouse at Glensaugh, were blood sampled on four occasions during late pregnancy. The primary object in the collection and analysis of these samples was to provide preliminary information required for a lactation experiment carried out by Peart. These prepartum data are, however, relevant in the present context. The relationships between birth-weight and the two plasma parameters determined (ketone and FFA concentrations) were significant at all four sampling dates. (These data are contained in Appendix 8). The correlation coefficients between plasma ketone concentration and birth-weight at six, four, two and one week before parturition were 0.48, 0.60, 0.77, and 0.81 respectively. Corresponding FFA: birth-weight correlations were 0.63, 0.65, 0.84, and 0.85. Intakes at these dates were respectively 16, 18, 20, and 21.3 g DOM per kg live weight.

These results indicate clearly that, within a group of ewes receiving comparable amounts of nutrients, the degree of undernourishment in individual animals is related to and dependent on foetal weight. They also suggest that, in the earlier studies on group-fed animals, the assumption of comparable individual intakes was valid, and that differences in the severity of undernourishment between individuals were a result of differences in foetal weight and not in nutrient intake.

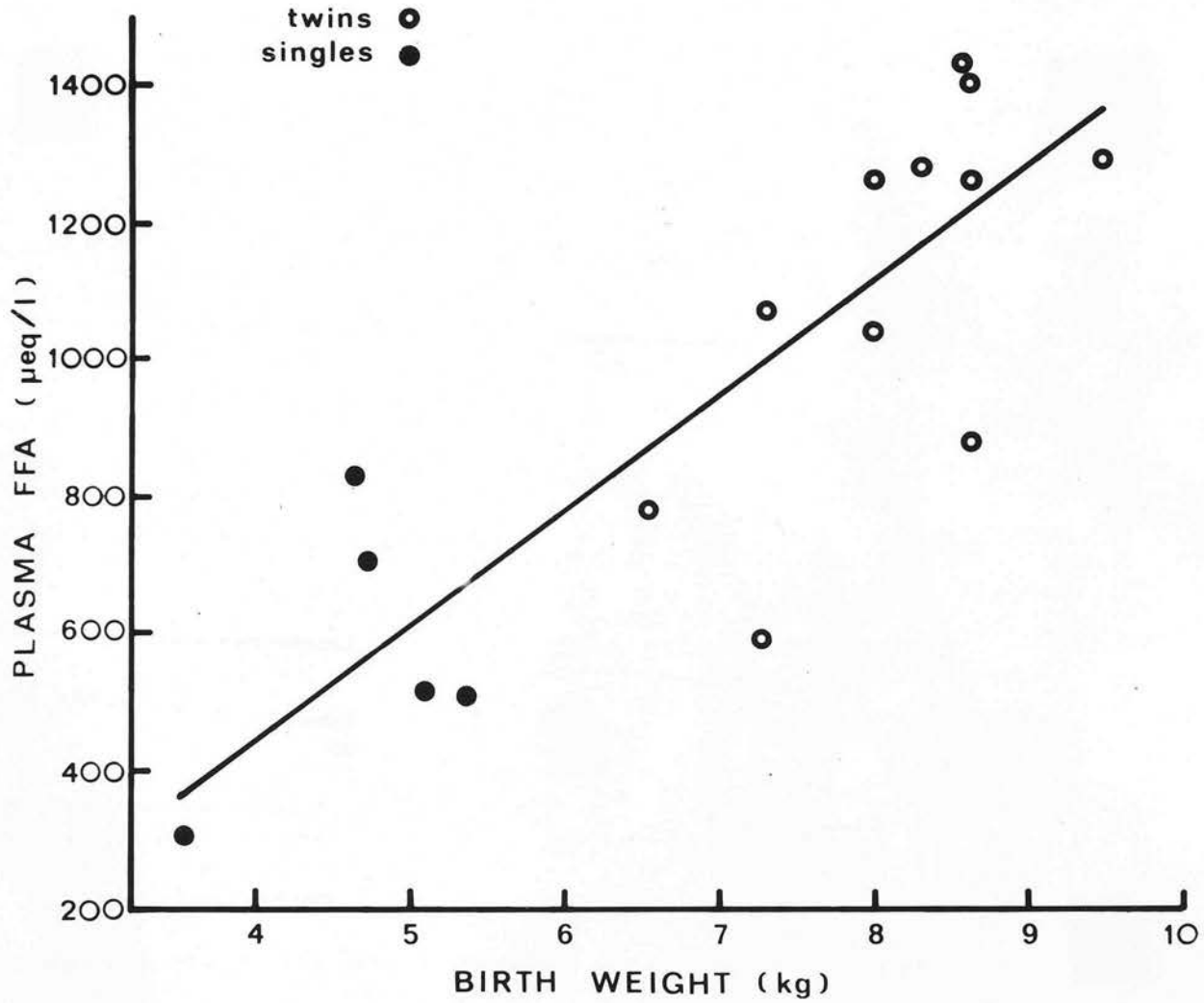

Fig. 10. The relationship between lamb birth-weights and plasma FFA concentrations during the final week of pregnancy in individually fed ewes with intakes of 21.3 g DOM per kg live weight.

The regression of plasma FFA concentration (μeq/l) (y) one week before parturition on birth-weight (kg) (x) in the group of ewes at Glensaugh was:

$$y = 167x - 228 \qquad r = 0.85$$

This relationship, which is illustrated in Figure 10, may be compared directly with that established from the data obtained from the ewes at Sourhope (Figure 8). Both sets of data refer to the same stage of pregnancy, and it is clear from a comparison of the results that the severity of undernourishment measured in the ewes at Sourhope was appreciably greater than that in the ewes at Glensaugh. Differences between the respective regression coefficients also indicate a relatively greater increase in the severity of undernourishment per unit increase in foetal weight in the ewes at Sourhope. These differences are not due to different requirements (foetal weights were considerably greater in the Glensaugh ewes, largely as a result of the large number of twins) and can only be a reflection of differences in intake. Although data on individual intakes were not available in the Sourhope study, the general level of intake was approximately one half of that in the Glensaugh ewes. A comparison of these two sets of results demonstrates the importance of level of intake as a factor determining the degree of undernourishment.

The results presented above constitute adequate evidence to confirm the hypothesis outlined at the beginning of this section, viz. that the degree of undernourishment in a ewe during late pregnancy is determined principally by foetal weight and the level of intake. The conclusions from these studies are that, within a group of

pregnant ewes with comparable intakes, the general degree of undernourishment is dependent on the level of the intake, and that the relative severity of undernourishment in individual animals is determined by differences in foetal weight.

4. Metabolic Responses to Induced Hypoglycaemia

It is pertinent at this stage to present and examine some data from one of a series of experiments on the comparative responses of ewes of different breeds to severe undernourishment. These data illustrate changes in and relationships between certain biochemical indices of nutritional state during the rapid development of artificially induced severe undernourishment.

The desired nutritional state was achieved in this instance by the use of the glucoside phloridzin (PHLORHIZIN), which produces a diabetic-like syndrome, but with a marked hypoglycaemia instead of the usual hyperglycaemia (Soskin and Levine, 1952). The drug produces its effect by inhibiting the phosphorylation of glucose to hexosephosphate. Phloridzin acts on all tissues, but is rapidly destroyed by muscular tissue. The kidneys are particularly susceptible to the action of phloridzin because of their limited ability to destroy the drug and because their excretory function leads to the accumulation of phloridzin in greater concentrations than elsewhere in the body. The action of phloridzin in the kidneys prevents the reabsorption of glucose by the kidney tubules, leading to losses of substantial amounts of glucose in the urine and hence to severe undernourishment, particularly when administered to a fasting animal. The loss of glucose in the urine of phloridzinized animals may be used to

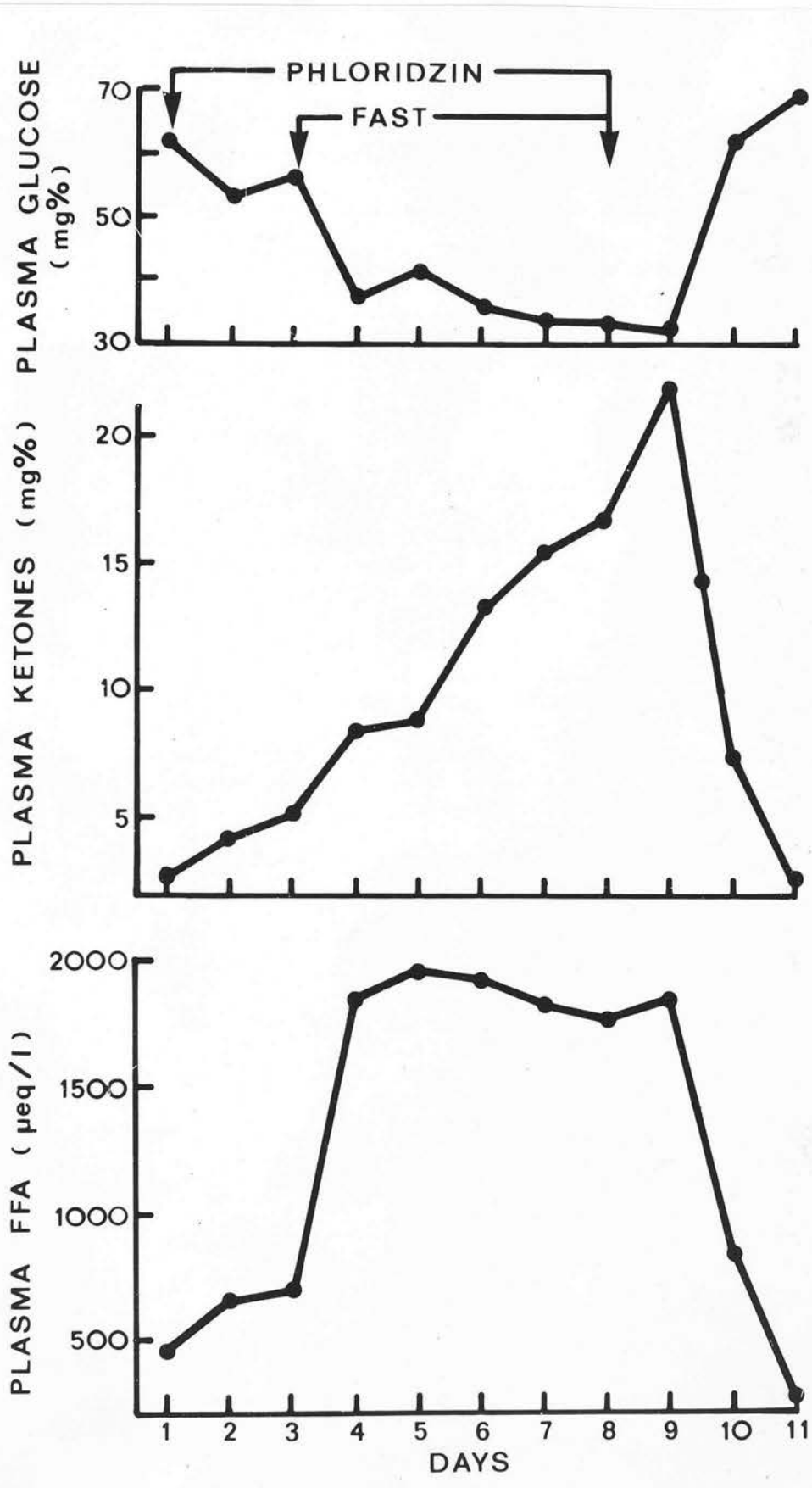

Fig. 11. The effects of phloridzin and fasting on mean plasma glucose, ketone and FFA concentrations in four Scottish Blackface ewes.

simulate the drain of glucose to the foetus in the pregnant ewe, and thus offers a means of reproducing, in non-pregnant animals, nutritional states which closely parallel those in late pregnancy. Evidence is contained in the work of Burtis, Jackson, Packett and Goetsch (1966), Jackson, Burtis and Goetsch (1966), and Reid (unpublished) that the effects of this particular drug in sheep are essentially the same as in the more commonly used momogastric laboratory animals.

Four non-pregnant Scottish Blackface ewes were treated with phloridzin (5 mg per kg, injected subcutaneously as a suspension in peanut oil) for eight consecutive days. The pre-experimental level of feeding of 10 g DOM per kg live weight was continued during the first two days of treatment, after which food was withheld until the day following the final phloridzin injection.

Mean glucose, ketone and FFA concentrations during and after the experimental period are illustrated in Figure 11. The daily urinary glucose excretions (see Appendix 9) increased from zero to 800 mg per kg live weight immediately after the first injection, and fell, on the withdrawal of food, to a level of approximately 350 mg per kg which was maintained until the end of the treatment period. Phloridzin had little effect on plasma glucose levels while the animals were being fed, but when feeding was discontinued these fell rapidly from approximately 55 to 35 mg %. Normal plasma glucose levels were regained within 24 hr of refeeding. (N.B. animals were fed on the ninth day immediately after blood samples had been collected).

Plasma ketone and FFA concentrations both showed moderate increases during the initial two days. When feed was withdrawn FFA concentrations increased from 700 to more than 1800 μeq/l within

24 hr, and remained at about this very high level until feeding was reintroduced. In contrast to this pattern, plasma ketone concentrations increased progressively during the six days of fasting, reaching a maximum of 22 mg % with no indication of any diminution in the rate of increase. The concentrations of both parameters decreased very rapidly on refeeding, and had returned to normal levels within 48 hr.

In fasting animals excreting approximately 20 g of glucose per day for six days, the severity of undernourishment must increase with time, yet only plasma ketone concentrations showed the progressive nature of the development of undernourishment. After fasting for 24 hr (i.e. 48 hr after the last food was given) plasma glucose and FFA concentrations gave no indication of any further increase in the severity of undernourishment. It is considered that by this time the rate of fat mobilization was probably at a maximum and that FFA concentrations had attained their upper limit. Glucose concentrations were low 24 hr after fasting, but not exceptionally so, and it is probable that adrenal hyperactivity reduced the rate of glucose utilization and prevented plasma concentrations falling even further, as suggested by Bassett, Mills and Reid (1966).

These data, those from early unpublished investigations by the writer, and results published by Reid and Hinks (1962c), indicate that plasma concentrations of both FFA and ketones increase in an approximately exponential manner, but at different rates, with increasing severity of undernourishment. Progressively developing undernourishment is reflected first by a small elevation in plasma FFA concentration which may not be accompanied by any detectable

change in the ketone concentration. At a later stage, when FFA are increasing very rapidly (i.e. at the steepest part of the response curve) the rise in ketone concentration is still comparatively slow, and it is not until FFA have reached their maximum that the rate of increase in ketone concentration becomes marked.

These results and the above considerations suggest that the two parameters in question may be used most efficiently as indices of different severities of undernourishment. Plasma FFA concentrations are more sensitive than ketone levels to changes in nutritional state at moderate levels of undernourishment, but in more severely undernourished animals, such as those treated with phloridzin while fasting, plasma ketone concentrations provide a better measurement of nutritional state.

One further point which must be noted at this stage, although implications will not be discussed until later, is the indication of a small decrease in plasma FFA concentration from approximately 1950 to 1750 μeq/l at a time when ketone concentrations were increasing rapidly.

5. The Use of Biochemical Parameters in Controlling Nutritional State

The conclusions drawn from results presented in earlier sections of this chapter led logically to an experiment which is the subject of this and the following two sections. The general approach and reasoning underlying the experimental design, which in many respects are as important as the results, are considered in the following chapter.

The objectives of this experiment were:

(i) to examine the possibility of using biochemical parameters to produce and maintain certain prescribed nutritional states, including two distinct degrees of undernourishment, in ewes during late pregnancy

(ii) to measure the effect of undernourishment during late pregnancy on lamb birth-weight, and

(iii) to estimate the energy requirements of the pregnant ewe.

Sixty Scottish Blackface ewes were mated at Glensaugh following progesterone treatment to synchronize oestrus. After mating in late November, the ewes grazed on sown pasture until early January, when they were randomly allocated to three treatment groups and penned individually, half of each group in the sheephouse, and half in outdoor pens. There were no detectable differences in any of the parameters measured within any group between the individuals in the outdoor pens and those in the sheephouse, and the results presented below have been computed from the pooled data collected in both locations.

A basal ration of hay was fed to all ewes from the time they were penned. This was increased progressively and later supplemented with the pelleted concentrate described earlier (Chapter IV, Section 3) as the nutrient requirements of the ewes increased. Where necessary the ratio of concentrate to hay was adjusted in such a way that total daily intakes did not exceed 30 g feed per kg live weight.

The method of feeding adopted for ewes in Group I was designed to ensure that all ewes were adequately nourished throughout the period of study. As the biochemical criteria employed in this experiment were indices of undernourishment and could not be used to assess the margin of nutrient intake in excess of requirement, the level of

feeding was not related to foetal size, but was designed to meet the highest requirement likely to be encountered. This was calculated from data published by Reid and Hinks (1962a, 1962b). An initial level of feeding of 20 g hay per kg was increased to 25 g per kg before the concentrate was introduced into the diet at 60 days prepartum. Thereafter the ratio of concentrate to hay was increased progressively, and the total offering maintained at 30 g per kg during the final six weeks of pregnancy.

In Group II the method of feeding was designed to produce and maintain a moderate degree of undernourishment, similar to that found in hill ewes with single foetuses, during the last six weeks of pregnancy. The investigations described in Section 2 of this chapter indicated that this particular nutritional state was characterized by plasma FFA concentrations of about 750 μeq/l. In an attempt to ensure that this degree of undernourishment was attained by six weeks prepartum the initial level of feeding in this group was 15 g hay per kg (approximately 7 g DOM per kg). This level of feeding was adjusted individually once a week to maintain as nearly as possible the prescribed plasma FFA concentration. Only those ewes with FFA concentrations in excess of 750 μeq/l at any particular sampling received an increase in the ration that week. No attempts were made to accelerate the onset of the desired degree of undernourishment by reducing the intakes of ewes with FFA concentrations less than 750 μeq/l. The intakes of such ewes remained unaltered until FFA concentrations increased as a result of increased foetal energy demands.

The object of the method of feeding adopted in Group III was to produce a relatively severe degree of undernourishment, characterized

by plasma ketone levels of 8 to 10 mg %, during the final six weeks of pregnancy. This particular level of undernourishment was considered to be typical of that found during late pregnancy in hill ewes with twin foetuses (Section 2). An initial submaintenance level of feeding of 10 g hay per kg (approximately 5 g DOM per kg) was adopted in an attempt to produce the desired degree of undernourishment within the prescribed time. Levels of feeding were adjusted individually once a week to maintain plasma ketone levels as nearly as possible within the prescribed limits. As in Group II individual adjustments were made only when the desired degree of undernourishment had been attained as the result of foetal growth.

In all three groups feed adjustments were calculated per kg live weight at the time of allocation of individuals to treatment groups.

Ad libitum feeding of the concentrate was employed in a further experiment during the post-partum period (Peart, 1967), and, to minimize any possible incidence of metabolic disorders following an immediate large increase in the ration, the amount of concentrate fed to all ewes was increased during the two days prior to the expected date of parturition.

All ewes were fed at 11.00 hr each day, and weighed and blood-sampled weekly, samples being collected before feeding. Plasma samples were prepared and frozen at Glensaugh and sent to Edinburgh by train for analysis. Some plasma FFA determinations and the preliminary stages of the glucose and ketone determinations were carried out on the evening of sampling. Full analytical results were available the following day, and feed adjustments were made at Glensaugh

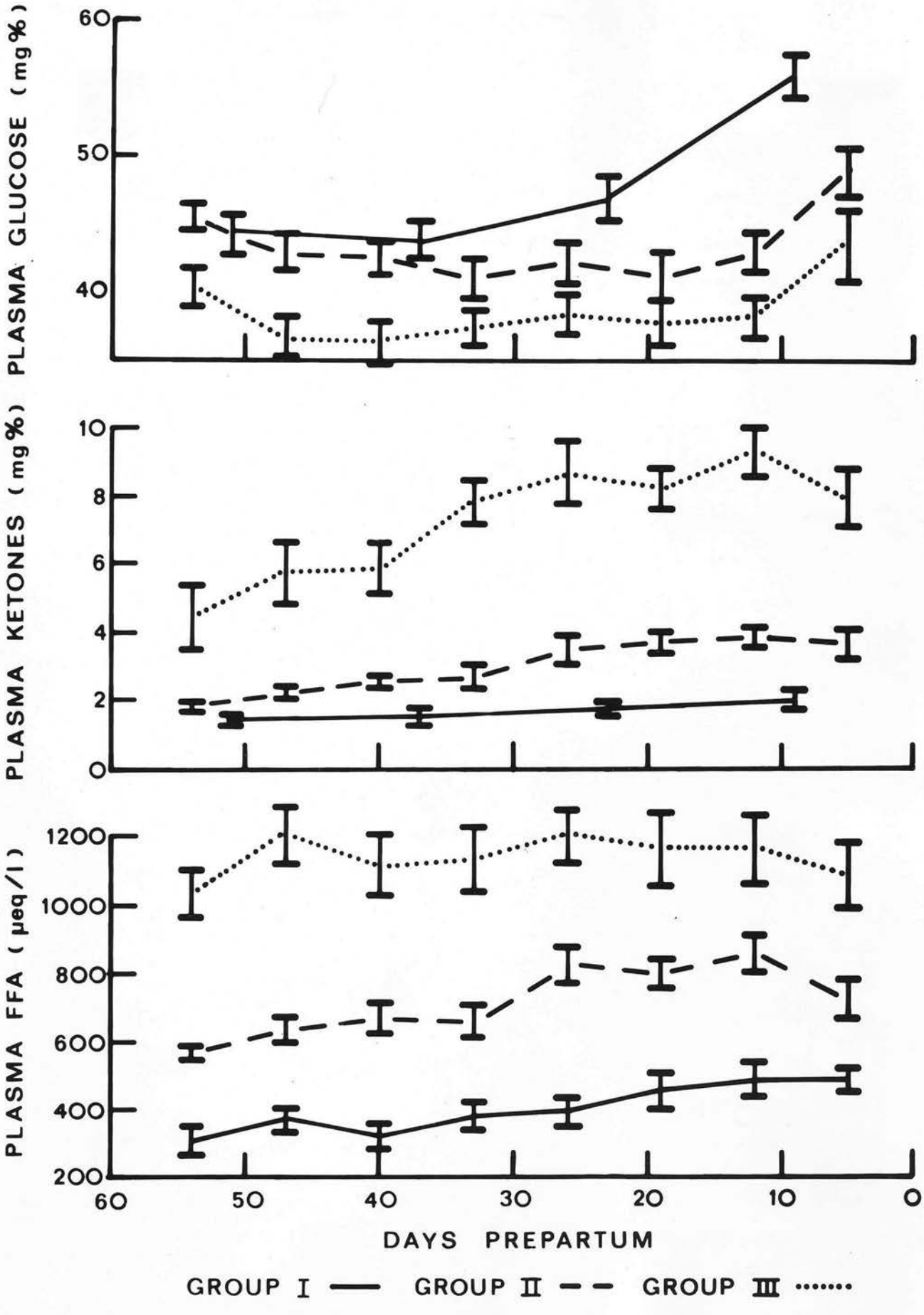

Fig. 12. Means and standard errors of plasma glucose, ketone and FFA concentrations in ewes of three treatment groups during late pregnancy.

in some instances within 24 hr, and always in less than 48 hr of blood sampling.

A number of ewes proved to be barren and some to have been mated at the second oestrus following synchronization. The data from these individuals have been excluded from the results presented here and in the following two sections. The 15 ewes in Group I which conceived during the first oestrus after progesterone treatment produced 9 single lambs and 6 pairs of twins, the 19 ewes in Group II produced 13 singles and 6 pairs of twins, and the 17 Group III ewes produced 8 singles, 8 pairs of twins and 1 set of triplets.

The amounts of feed supplied to ewes in Group I and the feed adjustments required to maintain ewes in the two undernourished groups in the prescribed nutritional states are more pertinent to the consideration of foetal requirements than in the present context, and are presented in Section 7 (Figure 14).

The metabolic responses, as measured by plasma glucose, ketone and FFA concentrations of the ewes in the three groups, to the treatments imposed are presented in Figure 12. (See also Appendix 10). In Group I mean plasma glucose concentrations up to four weeks before parturition were lower than would normally be expected. Plasma FFA concentrations were maintained below 500 μeq/l and ketones below 2 mg %, indicating that these ewes were not detectably undernourished at any stage.

Mean plasma FFA concentrations of ewes in Group II indicated that these ewes were measurably undernourished by 60 days prepartum, and that the severity of the undernourishment increased slowly thereafter until the prescribed level had been attained. Although the

predetermined degree of undernourishment was not achieved as quickly as had been hoped, mean plasma FFA concentrations were maintained within 100 μeq/l of the prescribed level throughout the last six weeks of pregnancy. These changes in plasma FFA concentration were accompanied by small but biologically meaningful changes in glucose and ketone concentrations.

In Group III ewes the mean plasma ketone concentration was significantly elevated by 60 days prepartum. Although the prescribed level of undernourishment was not fully achieved within the stated time, ketone concentrations were maintained within the prescribed limits during the final four to five weeks of pregnancy. By 60 days prepartum plasma FFA concentrations were in excess of 1000 μeq/l, and showed relatively little change from that time until parturition. The low plasma glucose concentrations in ewes of this group confirmed the greater severity of undernourishment indicated by the other parameters.

In all these groups there were small but consistent differences in the metabolic responses of single- and twin-bearing ewes, which indicated that, within each group, the ewes with heavier foetal weights were at a slight nutritional disadvantage relative to those ewes with lighter foetuses. The magnitude of the standard errors of the mean concentrations of the three plasma parameters (Figure 12) indicates, however, that these differences were small in relation to the differences between groups.

In general, the metabolic responses illustrated in Figure 12 indicate that the method of adjusting feed intakes according to the circulating concentrations of certain biochemical parameters can be

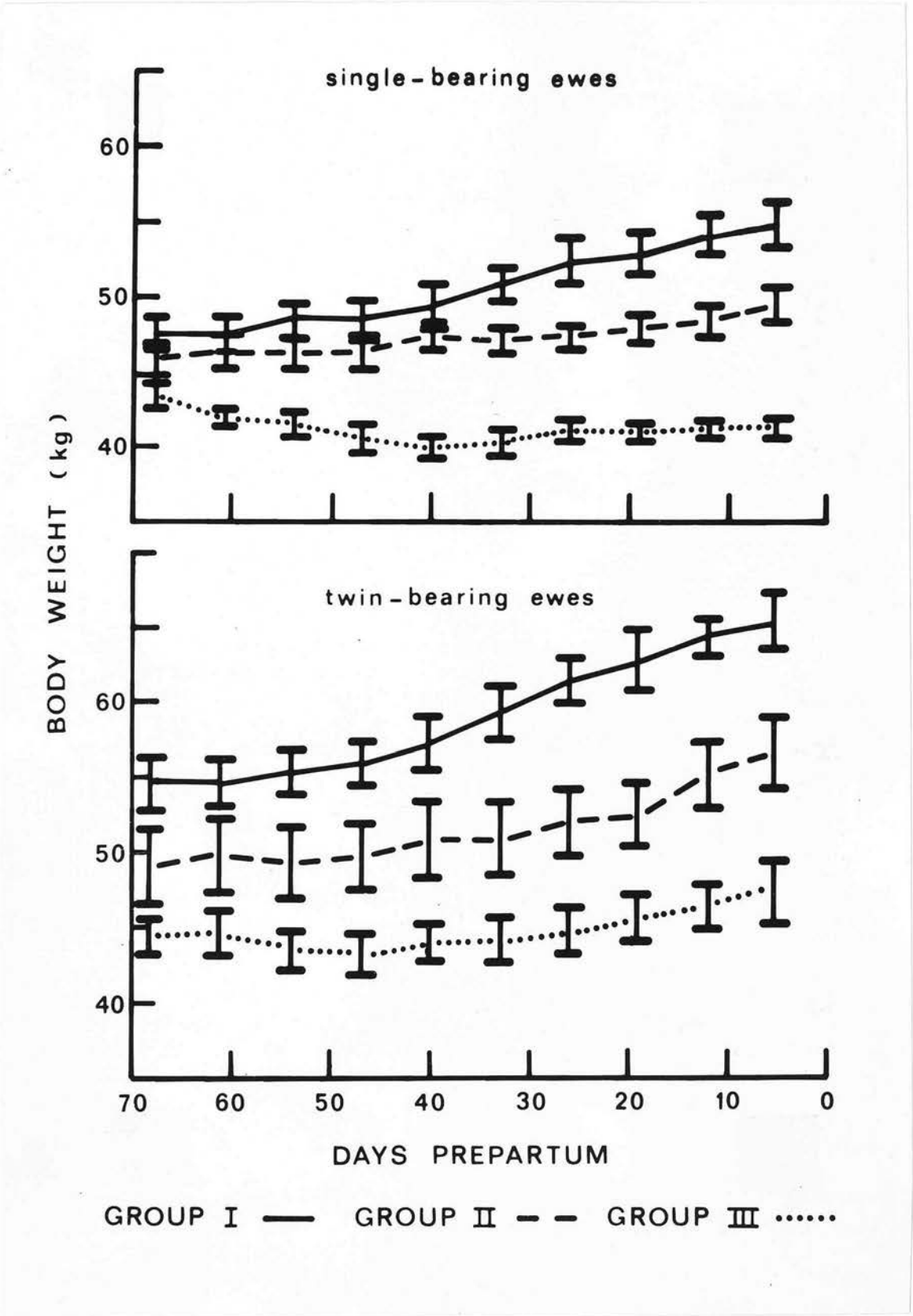

Fig. 13. Means and standard errors of live weights of single- and twin-bearing ewes in three treatment groups during late pregnancy.

used effectively to produce and maintain prescribed nutritional states in ewes during late pregnancy.

6. The Effect of Undernourishment During Pregnancy on Lamb Birth-weight

Mean live weights of single- and twin-bearing ewes in the three treatment groups described in the previous section are illustrated in Figure 13. The observed changes must be complicated to a variable degree by changes in gut fill as feed intakes increased with advancing pregnancy (see Section 7, Figure 14). Although data on the loss of live weight at parturition, which can be used as an estimate of the weight of total uterine contents, were not available in this experiment, it is clear that the gross live-weight increases of ewes in Group I were greater during the latter stages of pregnancy than the total increase in the weight of the gravid uterus as estimated from data presented by Thomson and Thomson (1949) and Reid and Hinks (1962a). This observation supports the biochemical evidence of the adequacy of feeding of ewes in this group. The severe degree of undernourishment in Group III ewes was reflected in the failure of gross live weight to increase significantly during late pregnancy in ewes with single foetuses, and in the very small but definite increase in ewes with twins.

The mean birth-weights of single, twin, and triplet lambs from ewes in the three treatment groups are presented in Table 5. There were inevitable differences within groups between the initial live weights of single- and twin-bearing ewes (cf. Thomson and Thomson, 1949; Wallace, 1961; Coop, 1962a; Reid and Hinks, 1962a). These

TABLE 5

Means and Standard Errors of Birth-weights

	Group I	Group II	Group III
Singles:			
Number	9	13	8
Birth-weight (kg)	4.6 ± 0.24	4.3 ± 0.18	3.5 ± 0.17
Birth-weight (g/kg)	101 ± 5.6	92 ± 4.7	76 ± 4.4
Twins:			
Number of pairs	6	6	8
Total Birth-weight (kg)	8.2 ± 0.49	7.1 ± 0.44	5.4 ± 0.40
Total Birth-weight (g/kg)	159 ± 7.6	144 ± 6.9	116 ± 7.5
Triplets:			
Number of sets	-	-	1
Total Birth-weight (kg)	-	-	7.5
Total Birth-weight (g/kg)	-	-	150

differences were corrected by expressing birth-weight in terms of the ewe live weights used in calculating feed adjustments.

The degrees of undernourishment experienced by the ewes in Groups II and III were associated with lower mean birth-weights of single and twin lambs. The moderate level of undernourishment in Group II reduced birth-weights of single lambs to 91% and of twin lambs to 90% of the respective weights in Group I. The more severe degree of undernourishment in Group III reduced birth-weights of single lambs to 75% and of twin lambs to 73% of the Group I weights. As these data suggest, the ratio of birth-weight of single lambs to the total weight of twin pairs remained relatively constant between groups (1 : 1.6 in Group I; 1 : 1.6 in Group II; and 1 : 1.5 in Group III), indicating that, within groups, ewes with twin lambs were no more severely undernourished than those with singles.

In measuring the effect of undernourishment during late pregnancy on lamb birth-weight the objective was not to add to the considerable evidence for such an effect which is already well documented in the literature (Table 1), but rather to assess the magnitude of the effect of certain measured degrees of undernourishment during late pregnancy, and to examine the comparative effects of such undernourishment on single and twin lambs. The demonstration of statistically significant differences at all levels requires a greater number of observations than are generally possible in an experiment of this type. Nevertheless, the reduction in birth-weight of both singles and twins, caused by the severe level of undernourishment, was significantly greater than that due to the moderate level of undernourishment ($P < 0.05$) and highly significant in relation to the birth-weights measured in Group I ($P < 0.01$).

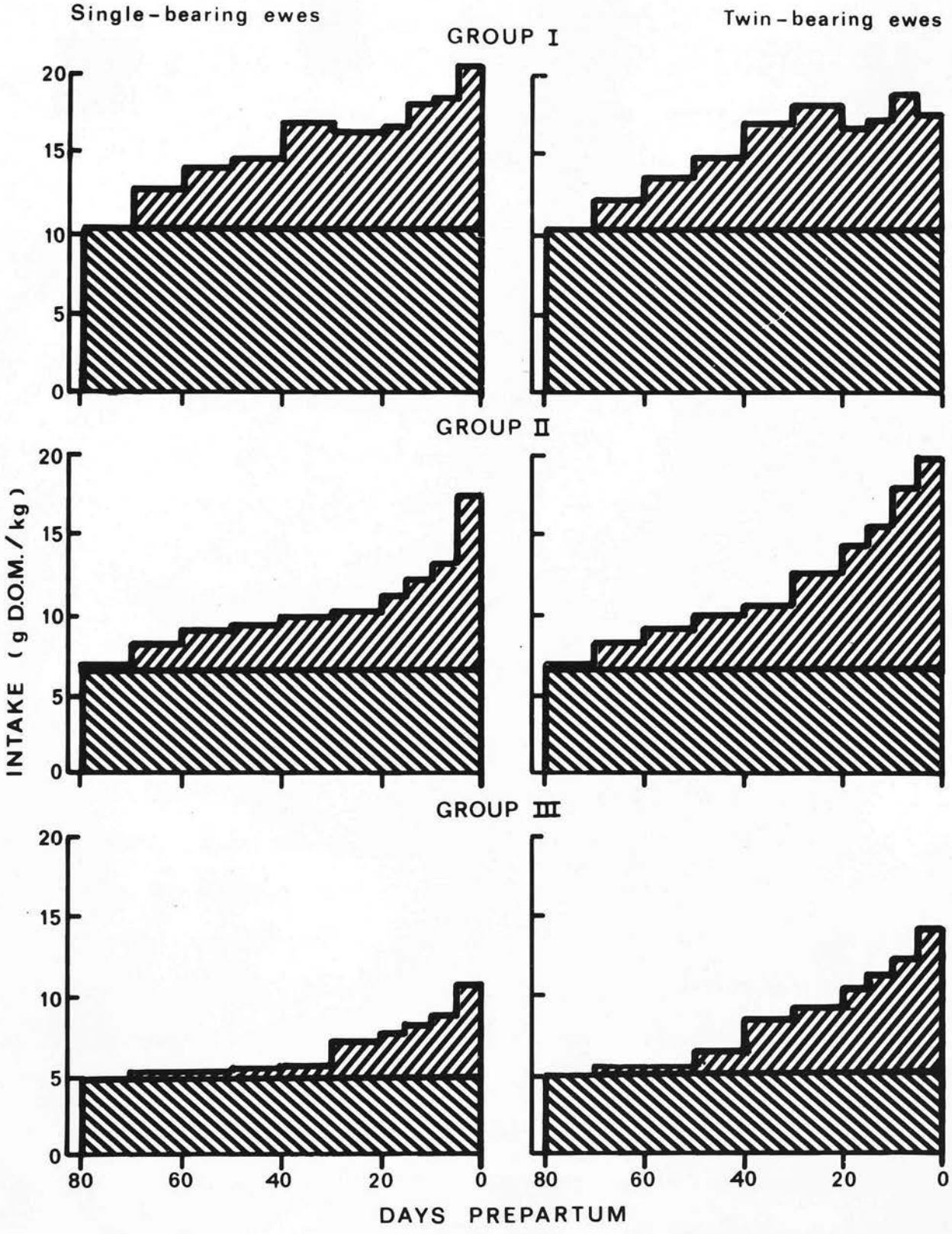

Fig. 14. Mean daily DOM intakes of single- and twin-bearing ewes in three treatment groups. (The lower portion in each diagram represents the basal level of intake; the upper portion shows the additional feeding supplied.)

These results provide a measure of the effect of certain specified nutritional states on lamb birth-weight, and suggest that a particular degree of undernourishment affects the birth-weight of single and twin lambs to the same extent.

7. Energy Requirements of the Pregnant Ewe

Mean daily intakes of hay and concentrates, expressed in terms of g DOM per kg live weight, of single- and twin-bearing ewes in the three groups described in Section 5, are illustrated in Figure 14. The increased amount of concentrates fed in anticipation of ad libitum feeding in the post-partum period is reflected in the intakes over the final five-day period.

The ewes in Group I, which served as a control against which the performance of other groups could be assessed, were well fed throughout the period of study at a level which the data in Sections 5 and 6 indicated to be in excess of the highest individual requirement. As these ewes were all fed the same arbitrary amount, per unit live weight, there was no real difference between the intakes of single- and twin-bearing ewes. Concentrates were always completely consumed, but small quantities of hay were frequently refused, especially when concentrates were added.

In Group II, in which intakes were individually adjusted according to plasma FFA concentrations, concentrates were first introduced at 60 days prepartum to those individuals whose hay intakes had by that time been increased from the basal 15 g per kg to 20 g per kg. Differences in the requirements of single- and twin-bearing ewes were established by 40 days prepartum and increased progressively

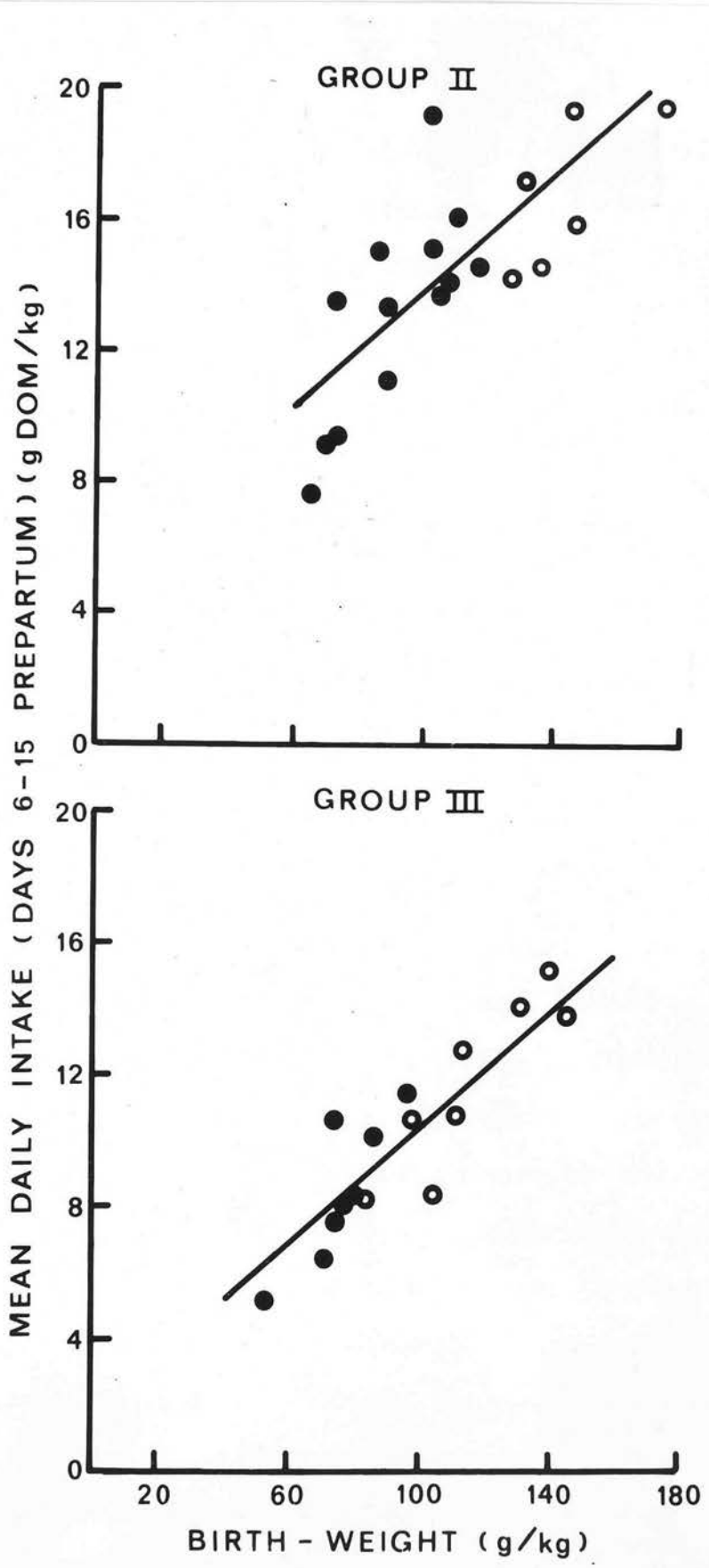

Fig. 15. Regressions of mean DOM intake 6-15 days prepartum of ewes in Groups II and III on foetal weight at term.

with advancing pregnancy. In the penultimate five-day period, i.e. before rations were increased in anticipation of ad libitum feeding, the mean daily intake of ewes with single foetuses was 15.3 g hay plus 9.2 g concentrates per kg, compared with 15.2 g hay plus 15.2 g concentrates per kg for the ewes with twin foetuses.

In ewes in Group III, in which intakes were adjusted to maintain a moderate ketosis, the basal 10 g hay per kg was increased as necessary to 15 g per kg, and further increases given in the form of concentrates which were first introduced to twin-bearing ewes at 40 days prepartum, and to single-bearing ewes at 30 days. There was no necessity, in this group, to reduce hay intakes at any time. In the penultimate five-day period the mean daily intake of single-bearing ewes was 14.4 g hay plus 1.9 g concentrates per kg. The corresponding intake of the twin-bearing ewes was 15.0 g hay plus 6.4 g concentrates per kg.

Relationships between feed intakes and lamb birth-weights were computed for Groups II and III. The intakes used in computation were those over the period 6 to 15 days prepartum. This particular period was chosen in preference to the final 10 days of pregnancy as it excluded the days immediately before parturition when intakes were increased in anticipation of post-partum ad libitum feeding. The regressions of mean daily intake over days 6 to 15, in g DOM per kg live weight (y), on total birth-weight, expressed as g foetus per kg live weight (x), are illustrated in Figure 15, and presented with the corresponding correlation coefficients below:

Group II	y	=	$0.087x$	+	4.94	r =	0.77
Group III	y	=	$0.087x$	+	1.58	r =	0.89

(The data on which these relationships are based are detailed in Appendix 11).

The correlation coefficients were both very highly significantly different from zero ($P < 0.001$), although not significantly different from each other. Deviations from regression were significantly less ($P < 0.05$) in Group III than in Group II.

The regressions of feed intake on foetal weight apply to ewes in a steady nutritional state, and the constant terms in the equations provide a theoretical measure of the DOM required to maintain non-pregnant animals in the particular nutritional states to which the equations apply. The regression coefficients provide an estimate of the daily foetal requirements at 6 to 15 days prepartum per unit weight of foetus at term. The curve of foetal growth calculated by Cloete (1939) indicates that the daily increase in foetal weight at this stage of pregnancy is of the order of 1.5%. If this rate of growth is in fact maintained by foetuses in undernourished ewes, the intakes used in computing the regressions apply to foetuses weighing 86% of the weight at birth. Applying this correction for growth during the final 10 days of pregnancy to the coefficients in the regression equations gives an estimate of the daily requirements of the foetus of almost exactly 100 g DOM per kg foetus.

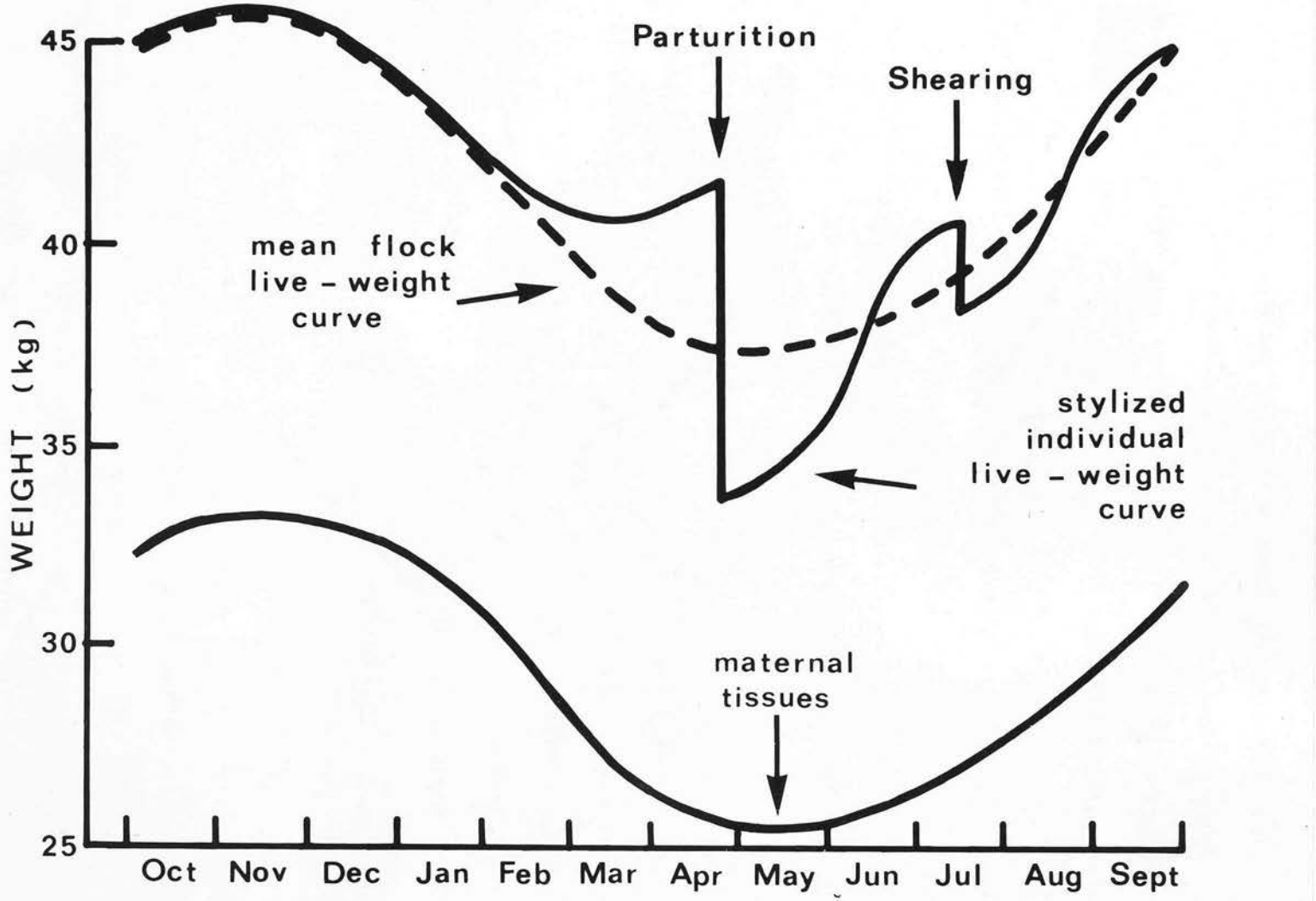

Fig. 16. Curve of mean ewe live weight, based on data in Figure 3, and stylized curves illustrating changes in individual live weight and maternal empty body weight during the year.

VI - DISCUSSION

1. Changes in Weight and Body Composition During Pregnancy

The data from the investigation of changes in the weight and body composition of hill ewes during pregnancy (Chapter V, Section 1) provide factual information regarding a well recognized, but poorly documented, situation.

In this investigation, which was conducted under conditions considered to be as representative of Scottish hill conditions as any single situation can be, the annual pattern of live-weight change (Figure 3) was similar to those computed from unpublished records (Figure 1) and those published by Robinson, Currie and Peart (1961). These all show an apparent difference of almost 20% between maximum and minimum live weights. They refer, however, to mean weights of large numbers of ewes in which lambing was spread over a period of several weeks. At about the time of parturition mean weights were based on varying proportions of pregnant and post-parturient ewes, and are therefore unlikely to reflect accurately the pattern of weight change in individual animals. In Figure 16 a stylized individual live-weight curve, computed from data presented in the previous chapter, is superimposed on the mean flock live-weight curve. This somewhat hypothetical individual live-weight curve is based on few data, but indicates clearly that the mean flock live-weight curve is likely to underestimate the extent of the live-weight loss in individual animals. It also illustrates the cyclic pattern of weight change during pregnancy, which results from the progressive decrease in weight of gastrointestinal contents

and the exponential weight increase of the contents of the gravid uterus. A stylized maternal empty body weight curve is included in Figure 16, and shows the increasing rate of loss of weight from maternal tissues which occurs as a result of the increasing deficit between the level of nutrient intake and the requirements of the ewe and foetus.

Fluctuations in live weight throughout the year are not unique to Scottish hill ewes; similar cyclic patterns are found in sheep in many different environments in other parts of the world. The unusual feature revealed by this study, however, is the low range of body condition over which the cycle occurs. The amount of fat present in the body at maximum live weight was 18% of the empty body weight, or only 13% of live weight, and at first sight scarcely constitutes an adequate reserve for a period of five to six months continuous weight loss.

Blaxter (1962) and Coop (1967) have drawn attention to the theoretical inefficiency of fluctuations in live weight and the use of body fat to buffer deficits between energy supply and demand. Energy is used more efficiently for maintenance purposes than for lipogenesis, and thus an animal which loses and subsequently regains body fat requires more energy over a given period of time than an animal which maintains a constant weight throughout. Economic considerations prevent this ideal situation being achieved under most free-grazing systems, and particularly under hill conditions, but, in exploiting the ability of the hill ewe to catabolize body reserves, this general principle of efficiency of feed utilization should not be disregarded.

Body fat is generally regarded as a concentrated reserve of energy which may be readily mobilized during periods of undernourishment. It is therefore somewhat surprising that the results show that during the first four months of pregnancy the weight of maternal tissue lost contained only a relatively small and statistically non-significant amount of fat, and a larger and significant amount of water. It must be borne in mind, however, that a large proportion of water loss emanates from the catabolism of muscular tissue, and that the 2.25 kg of water lost during the first period represents only about 0.5 kg protein. In terms of energy this is less than one sixth of the calories available from the relatively small amount of fat lost at this time. During the final month of pregnancy there was no apparent increase in the rate of protein catabolism, but fat mobilization increased approximately fourfold.

Although further studies involving the more frequent slaughtering of pregnant ewes are required to establish more precise patterns of fat and water loss, it is probable that the overall pattern is dependent on the initial fat content of the tissues before the period of undernourishment, as well as on the severity of the undernourishment. Panaretto (1964) found that prolonged undernutrition caused gradual depletion of fat and protein reserves of moderately fat Border Leicester x Merino ewes (less than 25% body weight as fat) until fat reserves were almost completely exhausted. Examination of Panaretto's data shows, however, that during the first half of the 201-day period of undernutrition, the weight of water lost from body tissues exceeded the weight of fat lost. A different pattern of tissue loss was noted in initially very fat ewes (fat

more than 40% body weight). In these ewes, 3 out of 4 of which died as a result of inappetence before fat reserves were depleted, the initial losses in body weight contained greater amounts of fat than water. Kirton and Barton (1958a, 1958b), who used l-thyroxine therapy and a low plane of nutrition in an attempt to reduce the fat content of overfat New Zealand Romney ewes (with more than 40% carcass fat), noted significant reductions in live weight and fat-free carcass weight, but found that neither treatment produced a significant effect on carcass weight, on the weight of fat in the carcass, or on the weights of omental and mesenteric fats. In a later experiment in which similar ewes (also with more than 40% carcass fat) were subjected to severe undernutrition, Hight and Barton (1965) could detect no significant effects of a 33 lb (15 kg) live weight loss on the weight of fat in the principal fat depots, although considerable water losses were demonstrated. Working with cattle, Butterfield (1966) noted that although substantial amounts of dissectible carcass fat were lost during 66 days of semi-starvation, the actual weight of muscular tissue lost was greater.

These results, and those from the experiment described in Chapter V, Section 1, suggest that in at least the earlier stages of weight loss the depletion of fat reserves may not be as great as is commonly supposed. This suggestion is, however, contrary to the findings of Robinson (1948) who recorded a decrease in the rate of fat loss in mature Border Leicester x Cheviot ewes on a submaintenance diet. This apparent anomaly may arise from the presumed increasing deficit between nutrient intakes and requirements in the present study and the constant submaintenance levels of intake in Robinson's experiment.

There is little information in the literature with which to compare the observations regarding the patterns of fat mobilization from different sites. The classical studies of growth and body composition in sheep, reviewed by Palsson (1955), record that undernutrition has an increasingly retarding effect on any tissue or tissues in the direct order of their maturity, i.e. latest maturing tissues are most affected. These studies generally describe fat deposition in terms of growth gradients similar to those demonstrated in muscle and bone, and not as a successive or proportionate accumulation of fat in different depots. Mention is also made, however, in other contexts, of subcutaneous fat as a late maturing tissue, and the early mobilization of fat from this depot and the late withdrawal of fat from bone, conform to the above hypothesis regarding tissue mobilization. However, the early losses of substantial amounts of water and smaller amounts of fat noted in this and other experiments referred to above are not in accordance with the pattern expected from results of these early studies.

In a report on the evaluation of body condition of free-ranging red deer in New Zealand, Riney (1955) described the order of deposition and mobilization of fat reserves. During periods of undernutrition the first depot to be mobilized was subcutaneous fat, followed by omental, mesenteric, perirenal and bone fats. This order of mobilization from the different sites agrees closely with the ranking of contributions to total weight of fat lost in the present study (Table 4). Classification of tissues according to the proportion of fat lost during the period of study (i.e. relative to initial weight of fat in each depot), however, gave a slightly

different ranking: subcutaneous, perirenal, omental plus mesenteric, that associated with musculature, remainder and bone.

The pattern of fat mobilization suggests that subjective assessments of body condition, in which subcutaneous fat is the most important single factor, may not provide as good an index of fat reserves as is commonly supposed, particularly at the lower end of the scale described by Jefferies (1961). Incidence of ewe mortality in hill flocks is greatest during the months when fat reserves are least (Gunn, 1967), but the actual mortality rate is nevertheless considerably lower than would be expected from Jefferies' observations on Merino ewes in a similar body condition (grade 1). If there is a difference in mortality rates between individuals of different breeds in the same subjectively assessed body condition, this could possibly be due to differences in the distribution of body fat; the Blackface may have more internal fat than the Merino at similar levels of subcutaneous fat. The lack of information in the literature regarding the distribution of fat in different breeds makes it difficult to find evidence for or against this suggestion, and indicates a need for further studies.

2. The Use of Biochemical Parameters as Indices of Undernourishment

The data presented on changes in weight and body composition show clearly that free-grazing hill ewes must be undernourished during late pregnancy. This section deals with the use of biochemical parameters to describe, in physiological terms, the severity of that undernourishment. The contributions of individual parameters in different situations are considered, and the implications of

certain physiological relationships are discussed in relation to some practical and theoretical problems.

(a) The uses of different parameters

The results of the investigations designed to characterize the nutritional state of free-grazing hill ewes during late pregnancy (Chapter V, Section 2) provide a good illustration of the use of certain biochemical parameters to describe, in physiological terms, naturally occurring nutritional states. The information supplied is more precise than measurements of body weight, and has the additional advantages that single samples from individual animals provide an indication of the direction of any change in body weight, and characterize the nutritional state of one individual in relation to that of other animals in the same group. The least and most severely undernourished animals in a group can be identified at any one point in time without recourse to measuring weight change, which may be complicated by differences in foetal size, over a period of time.

The pattern of metabolic responses to existing conditions and imposed experimental treatments described in the previous chapter are in general agreement with those established by other workers (e.g. Annison, 1960; Reid and Hinks, 1962a, 1962c; Patterson, 1963, 1964). As is to be expected from the metabolic relationships between these parameters, plasma ketone and FFA concentrations were positively related to each other, and were both negatively related to plasma glucose concentrations. The one equivocal result observed is the relatively low plasma glucose concentration of the adequately fed ewes (Group I) up to four weeks prepartum in the Glensaugh

experiment (Chapter V, Section 5). Ketone and FFA concentrations showed that these ewes were not undernourished. One possible explanation is that the increasing proportion of the concentrate diet in the intake of these ewes during the final six weeks of pregnancy led to an alteration in the pattern of VFA production in the rumen. This diet would be likely to favour the production of propionate, an important glucose precursor, to a greater extent than the hay diet which was reduced during this period.

In general, plasma ketone and FFA concentrations are the most useful, and hence most commonly used, indices of nutritional state. The primary cause of elevations in these parameters is a glucose insufficiency, which may be measured by changes in the rate of glucose utilization, but, as indicated in Chapter II, Section 5, is not necessarily reflected in significant changes in circulating glucose concentrations. The severe undernourishment caused by phloridzin treatment plus fasting (Chapter V, Section 4), and the three distinct nutritional states in the Glensaugh experiment (Chapter V, Section 5) can each be characterized by plasma glucose concentrations, despite the low values in one group mentioned above. In all of these instances, however, the other parameters provided at least as much, if not more, information regarding changes in nutritional state.

Although there is almost always a strong positive correlation between plasma concentrations of ketones and FFA, these parameters are not necessarily of equal value as indices of undernourishment. In certain situations one may prove a more appropriate criterion of nutritional state than the other. For example, in field situations, in which blood samples are collected from animals unaccustomed to the

procedure and handled without consideration of the possible effects of psychological factors on glucose and FFA concentrations, plasma ketone concentration is obviously the most useful, and indeed the only reliable parameter, even in instances of very moderate undernourishment in which ketone elevations are small. A similar degree of undernourishment in animals kept under closely controlled experimental conditions, and accustomed to frequent handling and routine blood sampling, would be more sensitively measured by circulating FFA concentrations.

The respective uses of ketone and FFA concentrations were considered briefly in relation to the results of the phloridzin experiment (Chapter V, Section 4). The conclusions from these and other results is that nutritional state is best characterized in terms of that parameter which shows the greatest response per unit change in either nutrient intake or nutrient requirement. The different rates of response of the two parameters to similar increases in the severity of undernourishment are such that plasma FFA concentration is the more sensitive index within the range of what has been termed moderate undernourishment; in severe undernourishment plasma ketone concentration is a better criterion than FFA level.

The above broad generalizations are, however, subject to certain qualifications. The first concerns the extreme lability of plasma FFA concentrations and their susceptibility to psychological factors, which has already been discussed. The second qualification concerns the apparent inability of animals to maintain very high FFA concentrations over prolonged periods. In the phloridzin experiment FFA concentration was at a maximum on the second day of fasting, and

thereafter appeared to decline slowly before dropping sharply on refeeding. The change during the last four days of treatment was not pronounced, but is similar to a response reported by Menahan, Schultz and Hoekstra (1966a) in fasted phloridzinized goats. These workers attributed this to a feedback mechanism preventing further rapid accumulation of ketone bodies in the blood. Recent studies by the writer on adaptation to prolonged severe undernourishment indicate that changes in thyroid activity may occur in such situations, and it is at least probable that one of the adaptive responses may be a lowering of metabolic rate. At present there is no conclusive evidence to support this particular hypothesis, but whatever the mechanism may be, it does appear that severely undernourished animals do not, or cannot, sustain maximum rates of fat mobilization for long periods.

It is probable that, in the severely undernourished (Group III) ewes in the experiment at Glensaugh (Chapter V, Section 5), plasma FFA concentrations were modified in the way suggested above, as these were not as high as might have been anticipated from the results of preliminary investigations. Whatever the cause, the results suggest that an attempt to maintain this particular level of undernourishment by adjusting feed intakes according to plasma FFA concentrations would have been less successful than that actually employed, which was based on ketone concentrations. The control of the moderate degree of undernourishment (Group II) on the basis of FFA concentrations was very satisfactory, and considered to be better than that which would have been achieved using ketone concentrations. The results of this experiment support

the suggestion made earlier that a moderate degree of undernourishment may be successfully characterized or controlled on the basis of plasma FFA concentrations, and that ketone concentration is the more useful criterion when dealing with relatively severe levels of undernourishment.

Although it is evident from the results of the Glensaugh experiment and from the above discussion that the technique of adjusting feed intakes according to biochemical indices of undernourishment was successful in producing and maintaining certain prescribed nutritional states during late pregnancy, the actual degree of control achieved should perhaps be examined more critically at this stage. The prescribed levels of undernourishment were not attained within the stated time. In both undernourished groups the majority of ewes had attained the desired nutritional state by six weeks prepartum but the development of undernourishment in the single-bearing ewes with small foetuses was appreciably slower. This suggests that the initial levels of feeding in these groups may have been slightly too high, and that the failure to achieve the prescribed degrees of undernourishment within the stated time could have been obviated by using lower initial levels of feeding.

There was also an indication, particularly in the two undernourished groups, that FFA and ketone concentrations in twin-bearing ewes were slightly but consistently higher than those of ewes with single foetuses. These differences presumably reflect the greater glucose requirements of twin foetuses, and the fact that, because feed adjustments were based strictly on the concentrations of the appropriate parameters and not on the number of foetuses a ewe was

thought to have, these adjustments were slightly and consistently less efficient in twin-bearing ewes. It should be borne in mind that the metabolic responses were measured at that part of the period between feed adjustments when differences between single- and twin-bearing ewes were likely to be greatest, and at the time of day when concentrations of ketones and FFA were likely to be highest. Diurnal variations in the levels of both parameters can be considerable (Reid and Hogan, 1959; Annison, 1960) and are likely to be greater in undernourished ewes with single foetuses than in those with twins. It should nevertheless be emphasised that the differences observed within groups between single- and twin-bearing ewes were very small in relation to the range of these parameters in undernourishment. The fact that within the two undernourished groups the birth-weight of twin lambs was reduced by the same extent as that of singles also indicates that differences in the degree of undernourishment between single- and twin-bearing ewes in the same group were negligible.

(b) Identification of twin-bearing ewes

The results presented in Chapter V, Section 3, showed that, in a group of pregnant ewes, the degree of undernourishment in any individual was determined by foetal weight and nutrient intake. Strong positive relationships between foetal weight and degree of undernourishment were demonstrated in several groups of ewes in which intakes of all ewes within each group were assumed, and in one instance known, to be comparable. In the regression equations presented, the degree of undernourishment was shown as being dependent on foetal weight, and although the results of the Glensaugh experiment demonstrated that birth-weight can be affected by the severity

of undernourishment, they also showed, by implication, that the dependent and independent variates in the regressions were correctly assigned, i.e. although the severity of undernourishment affects foetal weight, it is also determined by foetal weight.

Because the degree of undernourishment, as measured by biochemical parameters, has been shown to be dependent on foetal weight, it is not strictly legitimate, in the statistical sense, to use these parameters to predict foetal weight. There is, however, a good biological reason for ignoring statistical logic and transposing these variates. Any technique whereby the ewes with the heaviest foetal weights, which in most cases would be those with two or more foetuses, could be identified during late pregnancy would constitute a major advance in sheep production, by enabling more efficient use to be made of limited feed supplies.

A number of workers (Benzie, 1950, 1951; Ford, Clark and Gallup, 1963; Ardran and Brown, 1964) have investigated the use of radiological techniques in diagnosing pregnancy and determining the number of foetuses. In general, these workers have been able to make an accurate diagnosis of pregnancy 50 days or more after conception, and a more than 90% correct estimate of the number of foetuses at 90 to 110 days pregnant. On three separate occasions we have collaborated with an experienced radiologist in attempts to diagnose twin pregnancies in the field, and have been forced to conclude that, because the technique requires the use of X-ray and processing equipment outwith the scope of most field investigations, it has practical limitations under these conditions.

The data presented in Figures 7 and 9 (Chapter V, Section 3) provide convenient examples for examining the possibility of using biochemical indices of undernourishment to estimate foetal weight in late pregnancy, with the object of identifying twin-bearing ewes. In the Lephinmore investigation (Figure 7) there was no overlap in the distributions of birth-weights of single and twin lambs, but the distribution of plasma ketone concentrations of single- and twin-bearing ewes were not discrete, and it is thus not possible to differentiate clearly between these classes of ewes on this basis. The percentages and actual numbers of ewes with twin foetuses which would have been correctly identified by plasma ketone concentrations at each of the three sampling dates are shown in the second column in Table 6, assuming that the expected rate of twinning was in fact achieved. Selection on this basis of exactly the expected number of twin-bearing ewes would in fact have identified between 60% (second sampling) and 80% (first sampling) of all ewes with twin foetuses. Selection at this particular level would also have resulted in between 8 and 15% of the ewes with single foetuses being wrongly identified as having twins.

It is reasonable to suggest that the proportion of ewes selected for any preferential nutritional treatment should be higher than the expected rate of twinning. In this investigation the percentage of ewes with twins varied from 25 to 38.5% between the three samplings. Selection of the 50% of the ewes with the highest plasma ketone concentrations (Table 6, column 3) at the first or second sampling would have successfully identified 80% of all twin-bearing ewes. At the third sampling this level of selection would have been 100%

TABLE 6

Percentage of twin-bearing ewes correctly identified on basis of plasma ketone concentrations (cf. Figure 7)

(actual numbers shown in parentheses)

	Percentage of ewes selected	
	Expected percentage twins	50%
1st sampling 13 ewes (5 pairs of twins)	80 (4)	80 (4)
2nd sampling 18 ewes (5 pairs of twins)	60 (3)	80 (4)
3rd sampling 16 ewes (4 pairs of twins)	75 (3)	100 (4)

successful in identifying twin-bearing ewes, and would also have included 33% of ewes with single foetuses.

In the Castlelaw investigation (Figure 9) the distributions of birth-weights of single and twin lambs overlapped to a considerable extent, as did the distributions of the three plasma parameters of single- and twin-bearing ewes. The identification of ewes with twin foetuses would thus be expected to be less efficient than in the Lephinmore study. Again assuming that the expected rate of twinning was the rate achieved (39%), selection of exactly this proportion of ewes would have correctly identified between 57 and 62% of the twin-bearing ewes, depending on which parameter was used (Table 7). Increasing the proportion of ewes selected from 39 to 60% would have raised the efficiency of identification to between 81 and 86%. Although these figures are significantly better than the 39 and 60% efficiencies, which would result from completely random selection, it is doubtful whether they are sufficiently good to justify the use of this technique on a large scale.

In the example quoted above, in which three plasma parameters were measured, each parameter was used independently to judge which ewes were most severely undernourished. This does not require a knowledge of quantitative relationships between these parameters and foetal or birth-weights; ewes with the lowest plasma glucose concentrations or with the highest ketone or FFA concentrations are most likely to have the heaviest foetuses. If, however, relationships between these indices and birth-weight can be computed from existing data, then actual birth-weights can be predicted from each parameter independently, or from a combination of all three parameters.

TABLE 7

Percentage of twin-bearing ewes correctly identified on basis of three plasma parameters (cf. Figure 9)

(actual numbers shown in parentheses)

Parameter	Percentage of ewes selected	
	39%	60%
Glucose	62 (13)	81 (17)
Ketones	57 (12)	86 (18)
FFA	62 (13)	81 (17)

In a multiple regression, based on the data in Figure 9, of birth-weight (kg) (y) on plasma glucose (mg %) (x_1), ketone (mg %) (x_2) and FFA (μeq/l) (x_3) concentrations, all three independent variates provided significant predictive information regarding birth-weight. The actual equation was:

$$y = -0.03x_1 + 0.13x_2 + 0.001x_3 + 4.53$$

The standard error of estimate of this regression was $\pm$ 1.04 kg. The partial regression coefficients, which indicate the weighting to give to each parameter, and the regression constant will, of course, vary according to the nutrient intake of the ewes, and to the stage of pregnancy at which samples are collected. It is not legitimate to test this equation on the data from which it was derived, but the fact that each parameter contributed significantly to the prediction of birth-weight suggests that this combined quantitative approach would constitute a more efficient means of identifying twin-bearing ewes than selection on the basis of low glucose or high ketone or FFA concentrations.

One of the limitations of the use of this technique on a field scale could be the difficulty of obtaining blood samples from large numbers of ewes without the psychological disturbance generally associated with this procedure under normal conditions. The technique is also useful in selecting animals for experimental purposes, and the writer has used it on a number of occasions with considerable success. The technique can be used under controlled conditions to identify single- and twin-bearing ewes from about the nineteenth day of pregnancy, particularly if the severity of undernourishment is temporarily increased by withholding food for 24 to 36 hr before sampling.

(c) Considerations regarding experimental design

The conventional design of experiments, dealing with the effect of level of nutrition during pregnancy on subsequent performance, is based on two or more groups of ewes fed at different levels of nutrient intake. Some experiments incorporate major changes in the level of nutrition at certain critical times, and in others the level of nutrition is increased progressively with advancing pregnancy, but in almost all instances the intakes of all ewes within any group are the same, or are adjusted according to live weight or metabolic body size. The objectives of the nutritional treatments are generally to establish uniform nutritional states within groups, and differences in nutritional states between groups.

The results presented in the previous chapter indicate that this type of design will create the desired differences in nutritional state between groups (i.e. between the average degrees of undernourishment of each group), but will almost certainly result in large variations in nutritional state within groups, because of the dependence of degree of undernourishment on foetal weight, as well as on nutrient intake. These variations within groups are frequently considerable in relation to between group differences. For example, in an experiment incorporating three levels of intake, the differences between the 'high' and 'medium' and between the 'medium' and 'low' levels are likely to be of the order of half a pound of concentrates per day, i.e. equivalent to about 150 g DOM. In this example the largest difference in intake (i.e. between the 'high' and 'low' level ewes) is 300 g DOM. The difference in requirements, during the final stages of pregnancy, between a ewe with a small single foetus

weighing, say, 3.5 kg, and another ewe with twin foetuses totalling, say, 8.5 kg, will be 500 g DOM (Chapter V, Section 7). If both ewes are in the same group the difference in their nutritional states will be equivalent to 500 g DOM. If, however, the ewe with the small single foetus is in the 'low' group, and the other in the 'high' group, the 'poorly' fed ewe will have a nutritional advantage equivalent to 200 g DOM over the 'well' fed ewe.

The above example is not intended as a criticism of the many valuable experiments referred to in Table 1; the higher nutrient requirements of twin-bearing ewes have been demonstrated in a number of these experiments (e.g. Underwood, Shier and Cariss, 1943; Guyer and Dyer, 1954; Gardner and Hogue, 1963). The example, however, puts the likely variations in the severity of undernourishment within and between groups into perspective, and shows that in relation to differences between groups, the variation within groups may be considerably greater than has been generally appreciated.

Another feature of the conventional experimental design which is not wholly satisfactory, and which is related to the within group variation in nutritional state, is the effect of undernourishment on lamb birth-weight. In a group of ewes which are adequately nourished during pregnancy (i.e. in which energy intakes equal or are greater than requirements) lamb birth-weights will, for non-nutritional reasons, be normally distributed over a wide range. In the 'medium' or 'low' plane groups in the above example, however, ewes with the largest foetuses will be most severely undernourished, and the birth weights of their lambs will be affected to a greater extent than those of ewes with smaller foetuses. The distributions of birth-

weights in these groups is likely to be affected in such a way that it may not be possible to demonstrate a statistically significant effect from standard comparisons of group means. In these circumstances the experimental treatment affects some ewes more than others and may have no effect whatsoever in a number of individuals, yet the effect of the treatment is judged on the mean performance of the whole group.

Clearly any technique whereby all individuals within a treatment group can be maintained in the same prescribed nutritional state offers a better means, not merely of demonstrating that undernourishment during late pregnancy affects birth-weight, but of measuring the extent of the effect of specific nutritional states on subsequent productive performance, as judged in terms of birth-weight and other parameters of production. The technique of adjusting individual feed intakes according to certain biochemical indices of undernourishment offers a rational means of exercising the necessary control over nutritional state. This approach may not duplicate the naturally occurring situation on which the conventional experimental design, with comparable intakes within groups, is based, but it is considered that it is better suited for studying the effects of nutrition during pregnancy on subsequent performance.

3. The Effect of Undernourishment During Late Pregnancy on Lamb Birth-weight

The results presented in Chapter V, Section 6, indicate that even moderate undernourishment during late pregnancy causes a reduction in lamb birth-weight. The extent of this reduction is directly related to the degree of undernourishment which, under conditions of

comparable nutrient intakes, is dependent on the weight of the foetus or foetuses that a particular ewe is carrying.

The two levels of undernourishment in the Glensaugh experiment were selected, on the basis of the field investigations at Lephinmore (Chapter V, Section 2) as being typical of the nutritional states found in 'average' single-bearing and 'average' twin-bearing hill ewes during late pregnancy, in so far as any particular nutritional state can be representative of a naturally occurring population. Bearing this qualification in mind, it can be suggested that the undernourishment which occurs in hill ewes with single foetuses may be expected to cause a reduction in birth-weight of some 10% compared with that which could have been achieved under conditions of optimum nutrient intake. It is unlikely that a reduction in birth-weight of this order will have a sufficient effect on lamb survival or growth rate to justify the input required to prevent it. The severe degree of undernourishment which occurs during late pregnancy in hill ewes with twin foetuses causes a reduction in birth-weight of approximately 25%, and although in the Glensaugh experiment there were no instances of lamb mortality which were in any way attributable to the experimental treatment imposed, it is to be expected from Alexander and Peterson's (1961) studies that the survival of twin lambs from severely undernourished ewes would be prejudiced under hill conditions.

Blaxter (1962) has noted that (the weight of) the foetus of the pregnant ewe appears to be extremely sensitive to the effects of energy supply. It is difficult, however, to find data in the literature relating to large mammals with which to compare effects noted in the sheep.

Experiments and investigations by Blaxter (1944), Bonsma (1949) and Braude and Walker (1949), which have been reviewed by Blaxter (1957), demonstrated effects of level of maternal nutrition on birth-weight in cattle. The magnitude of these effects was, however, less than that recorded in sheep in the classical studies of Wallace (1948a).

Hytten (1963) and Hytten and Leitch (1964) have considered birth-weight data from human infants born during famine. During the seige of Leningrad in 1941 and 1942 nutritional conditions were particularly severe, and Antonov (1947) reported that the average birth-weight fell by a maximum of 600 g. In the Dutch famine of 1945 Smith (1947) calculated that the average birth-weight fell by about 240 g, although most pregnant women in Rotterdam gained only approximately 2 kg and many lost weight. Hytten (1963) points out, however, that the most striking consequence, in reproductive terms, of famine conditions is amenorrhoea and infertility, so that the relatively few babies that are born represent a highly selected group which cannot be compared accurately with data obtained before the famine.

In comparing the effect of undernourishment during late pregnancy on birth-weight in different species it should be borne in mind that few species are subjected to the severe dietary restrictions regarded as commonplace in many breeds of sheep, and that in these other species the incidence of multiple births is generally lower.

4. Energy Requirements and Utilization in the Pregnant Ewe

A criticism which may be levelled with some justification against the estimates of foetal requirements presented in Chapter V, Section 7, is that no account has been taken of possible associative effects on the digestibility and utilization of the two feeding stuffs used in the experiment. In designing the experiment it was recognized that the use of a single diet of sufficiently high quality to ensure the prevention of undernourishment in the Group I ewes would necessitate levels of intake in Group III ewes which could not be tolerated on humanitarian if not physiological grounds. The combination of hay and concentrates was chosen and manipulated to ensure a reasonable degree of rumen fill in the severely undernourished ewes, and the ingestion of more than adequate amounts of nutrients in the well-fed ewes. While recognizing that associative effects almost certainly occurred, it is unlikely in feeding hay and a pellet based on dried grass that such effects materially affected the results. In a discussion of these effects Blaxter (1962) considers that "after a period of time ruminants adapt to mixed diets, and that associative effects tend to be small provided sufficient time has elapsed to enable the microflora of the rumen to readjust to its changed substrates".

The daily foetal requirement of 100 g DOM per kg foetus, calculated from the results of the Glensaugh experiment, is equivalent to an energy requirement of 365 kcal ME per kg of foetus. In an experiment by Reid and Hinks (1962a), in which feed intakes were adjusted on the basis of plasma ketone concentrations to prevent

undernourishment during late pregnancy, Reid (1963) calculated the daily foetal requirements as 320 kcal ME per kg. In discussing the use of different biochemical parameters as criteria of undernourishment, Reid and Hinks (1962b) expressed the opinion that plasma FFA concentration was a more sensitive index than ketone concentration, and concluded that if feed adjustments had been made on the basis of FFA concentrations instead of ketones, their estimate of foetal energy requirements (Reid and Hinks, 1962a) would have been greater. It is thus likely that Reid's (1963) figure is an underestimate, and that the actual requirement was of the same order as that obtained in the Glensaugh experiment.

The above estimate of foetal energy requirement must be subject to alteration according to the quality of the diet. It is difficult to assess the ME content of the diets on which the above estimate is based because of wide variations in the proportions of hay and concentrate fed to different individuals in the two undernourished groups. It is likely, however, that the energy concentrations, i.e. the $^{M}/_{D}$ values referred to by the Agricultural Research Council (1965), of individual intakes were of the order of 2.3 to 2.6 Mcal ME per kg dry matter.

The results quoted above cannot be compared directly with the requirements given by the National Research Council Committee on Animal Nutrition (1957, 1964), whose figures are based on ewe body weight and not on foetal weight. This is, of course, the more meaningful way to express practical recommendations, but in comparing these figures with experimental results it is necessary to make certain assumptions regarding maternal maintenance requirements and

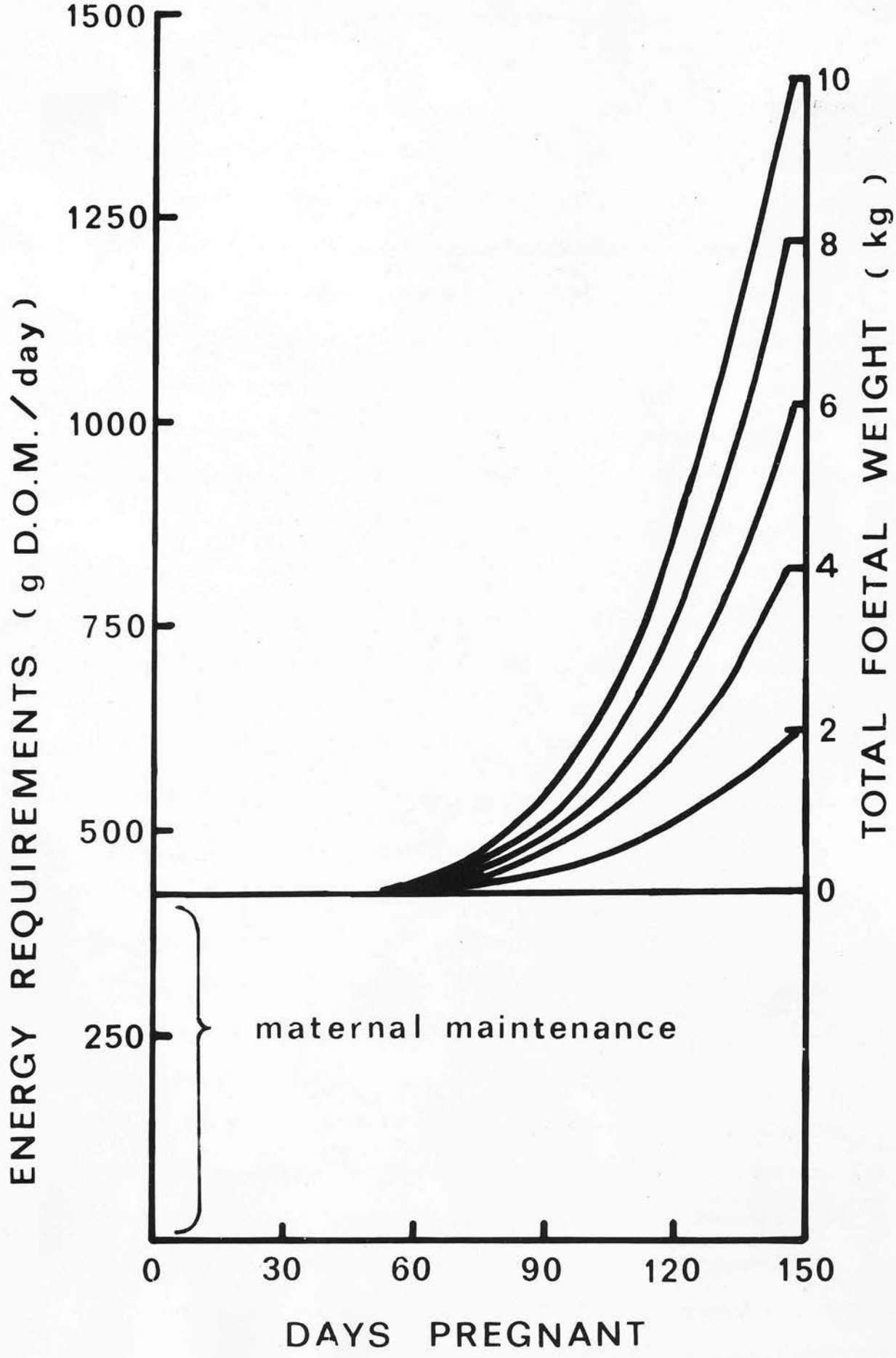

Fig. 17. Theoretical amounts of energy required to prevent undernourishment during pregnancy in 50 kg ewes carrying different weights of foetus.

average foetal size. Calculations based on the above foetal requirement, and using ewe and foetal weight data from the Group 1 (adequately fed) Glensaugh ewes, show that the National Research Council recommendations would provide almost 100% of the total requirements of single-bearing ewes and about 75% of the requirements of twin-bearing ewes at the end of pregnancy. This observation regarding the adequacy of the National Research Council recommendations is in general agreement with the findings of Whiting and Slen (1958), Smoliak and Slen (1958), Wright, Pope and Phillips (1962) and Gardner and Hogue (1963).

Assuming a maternal maintenance requirement of 8 to 9 g DOM per kg (Coop, 1962b; Lambourne and Reardon, 1963; Langlands, Corbett, McDonald and Pullar, 1963a, 1963b), it can be calculated that a daily intake of 900 g DOM in late pregnancy would prevent undernourishment in a 50 kg ewe with an average sized single foetus, but would cause a relatively severe degree of undernourishment, with a moderate ketosis (plasma ketone concentration of about 10 mg %), in a similar ewe with twins. The complete relief of this degree of undernourishment would require a further 350 g DOM per day. To produce a similar degree of ketosis in a single-bearing ewe, DOM intake would require to be reduced to 500 g per day.

Energy intakes required to prevent undernourishment during late pregnancy in ewes carrying foetuses of different weights are illustrated in Figure 17. These requirements, calculated from results with penned sheep, are considerably in excess of possible intakes of pregnant ewes on hill grazings where maintenance costs are likely to be higher. Studies by Eadie (1967) of the annual cycle of

nutrient intake suggest that, for the greater part of pregnancy, DOM intake is of the order of 350 to 500 g per day, rising to 700 to 800 g during the month before parturition.

It is important to emphasize that the high levels of energy requirement referred to above exist only for a relatively short period, as illustrated in Figure 17, and represent levels of intake which are necessary to completely prevent undernourishment during late pregnancy. This does not imply that management systems should be altered in an attempt to completely avoid undernourishment. The results discussed in the first section of this chapter show that the hill ewe is well able to catabolize body reserves laid down at times of plentiful nutrient supply to meet high energy requirements during periods of enforced low nutrient intake.

The shape of the nutrient requirement curves in Figure 17 suggests that, in management systems dependent on an input of supplementary feeding, a limited quantity of feed is better given in progressively increasing amounts over the last three to four weeks of pregnancy than at a constant rate over the final eight weeks, as is frequently the practice. A total input of, e.g. 28 lb concentrates per ewe is likely to have a greater effect given as, say, $\frac{1}{2}$ lb per day during the fourth week prepartum, $\frac{3}{4}$ lb per day during the third week, $1\frac{1}{4}$ lb per day during the second week, and $1\frac{1}{2}$ lb per day during the final week, than distributed over eight weeks at $\frac{1}{2}$ lb per day. The latter amount represents a daily input of only about 150 g DOM, which is relatively little in relation to later requirements.

The high foetal requirements for energy suggest that the utilization of energy for foetal growth must be relatively inefficient.

Graham (1964) estimated foetal maintenance requirements as 90 kcal ME per kg per 24 hr. Subtracting this from the total requirement of 365 kcal per kg leaves 275 kcal per kg per 24 hr available for foetal growth. From Graham's data it can be calculated that in late pregnancy the daily energy storage in the foetus is of the order of 20 kcal per kg. Thus the net efficiency of energy utilization by the foetus is about 7%, or half that calculated by Graham. Even allowing that this may be an underestimate, it is evident that the energy cost of reproduction is considerably greater than that of other types of production.

The Agricultural Research Council (1965) do not give any estimate of the nutrient requirements of the pregnant ewe, but in discussing the efficiency of energy utilization for foetal growth in cattle, they note that heat production increases in late pregnancy at a rate which is greater than would be expected for a non-pregnant animal retaining the same amount of energy. They continue: "Whether it is legitimate to attribute part of the increase in the heat production of the fed animal to a low efficiency of the processes concerned in the deposition of new material in the uterus is doubtful. In dealing with pregnancy it appears to be more simple to assume that it is the maternal maintenance cost which changes and that the energetic efficiency of the gains is the same as in normal growth".

It is not possible from the data collected in the Glensaugh experiment to make any estimates of changes in maternal maintenance costs during pregnancy in sheep, but examination of the results suggest that, if any changes did occur, these were more likely to

be in a negative than a positive direction. The calculations involved are of doubtful validity, and although perhaps allowable in the context of a thesis, are not considered to be sufficiently well founded for purposes of wider publication.

As indicated in Chapter V, Section 7, the constant terms in the regression equations of feed intake on birth-weight (Figure 15) provide a theoretical measure of the energy required to maintain non-pregnant ewes in the particular nutritional states to which the equations apply. Ewes in both groups for which equations were given, were undernourished (i.e. fed at levels less than that necessary to meet requirements), and those in Group I were fed in excess of requirements. If the intakes of these Group I ewes had been adjusted in such a way that undernourishment was just prevented (based, e.g. on plasma FFA concentrations of 500 μeq/l), the constant term in the appropriate regression would have provided an estimate of maternal maintenance requirements in adequately nourished pregnant ewes. Although this particular treatment was not included in the experiment it is not unreasonable to argue that the regression would have been parallel to and above the two regressions actually derived. It can also be reasoned that the upward displacement of this regression would have been in proportion to the differences in mean birth-weight between groups. If the difference between the regression constants in Groups II and III is equivalent to a birth-weight difference of 15% (Chapter IV, Section 6) it can be calculated that the constant of the hypothetical regression would have been 7.2 g DOM per kg ewe body weight.

This method of calculation is open to criticism, but the estimate, which is somewhat lower than those of Coop (1962b), Lambourne and Reardon (1963) and Langlands, Corbett, McDonald and Pullar (1963a, 1963b) used elsewhere in this thesis, is not unreasonable. Assuming an efficiency of utilization of ME of 70%, it can be calculated from the Agricultural Research Council (1965) preferred values for fasting metabolism that the maintenance requirement of a four-year-old 50 kg non-pregnant sheep is 7.5 g DOM per kg. This figure does not include an allowance for the energy cost of activity which would probably increase this estimate by 0.5 to 1.0 g DOM per kg (Agricultural Research Council, 1965). It is of interest to note that Thomson and Aitken (1959) considered that in pregnancy the efficiency of energy utilization is increased. This implies a lower maternal maintenance cost. Any metabolic adaptation to a less than optimum nutrient supply, such as the decrease in thyroid activity mentioned briefly in Section 2(a) of this chapter, would also be likely to lower, rather than raise, maternal maintenance requirements.

None of the evidence on this point is in any way conclusive, and indeed most of it is at present little more than conjecture, but it does suggest that the assumption regarding the partition of nutrients in pregnant cows, as stated by the Agricultural Research Council (1965), does not necessarily apply to pregnant ewes.

VII - SUMMARY

1. An examination of changes in weight and body composition in a flock of free-grazing Scottish Blackface ewes indicated that, during the greater part of pregnancy, these ewes were undernourished and catabolizing considerable amounts of body tissue.

2. The nutritional states of ewes from two differently managed Scottish Blackface flocks were characterized during late pregnancy in terms of circulating concentrations of plasma free fatty acids and ketones. Marked undernourishment was evident in both situations.

3. Data collected in a variety of situations supported the hypothesis that the severity of undernourishment during late pregnancy is determined principally by foetal weight and the level of food intake. Within a group of pregnant ewes with comparable intakes, the general degree of undernourishment is dependent on the level of intake, and the relative severity of undernourishment in individual animals is determined by differences in foetal weight.

4. The use of certain biochemical parameters as indices of undernourishment was examined in ewes with artificially induced hypoglycaemia. It was concluded that nutritional state is best characterized in terms of that parameter which shows the greatest response per unit change in either nutrient intake or nutrient requirement.

5. Biochemical parameters were used to control the nutritional states of individual animals in an experiment on the effects of undernourishment during pregnancy on lamb birth-weight. The results of this experiment indicated that the undernourishment occurring in free-grazing hill ewes during late pregnancy was likely to reduce the birth-weight of single lambs by 10%, and that of twins by 25%.

6. The additional energy requirements during pregnancy were estimated to be 100 g digestible organic matter per kg foetus.

VIII - REFERENCES

AGRICULTURAL RESEARCH COUNCIL. 1965. The Nutrient Requirements of Farm Livestock. No. 2 Ruminants. Agricultural Research Council, London.

ALEXANDER, G. and PETERSON, J.E. 1961. Neonatal mortality in lambs. Aust. vet. J. 37 : 371-381.

ALEXANDER, R.H. and McGOWAN, M. 1961. A filtration procedure for the in vitro determination of digestibility of herbage. J. Br. Grassld Soc. 16 : 275-276.

ANNISON, E.F. 1960. Plasma non-esterified fatty acids in sheep. Aust. J. agric. Res. 11 : 58-64.

ANNISON, E.F. 1965. Absorption from the ruminant stomach. In Physiology of Digestion in the Ruminant (ed. R.W. Dougherty, R.S. Allen, W. Burroughs, N.L. Jacobson and A.D. McGilliard), Butterworth, London.

ANNISON, E.F., HILL, K.J. and LEWIS, D. 1957. Studies on the portal blood of sheep. 2, Absorption of volatile fatty acids from the rumen of the sheep. Biochem. J. 66 : 592-599.

ANNISON, E.F., LENG, R.A., LINDSAY, D.B. and WHITE, R.R. 1963. The metabolism of acetic acid, propionic acid and butyric acid in sheep. Biochem. J. 88 : 248-252.

ANNISON, E.F. and LEWIS, D. 1959. Metabolism in the Rumen. Methuen, London.

ANNISON, E.F. and LINDSAY, D.B. 1962. The measurement of entry rates of propionate and butyrate in sheep. Biochem. J. 85 : 474-479.

ANTONOV, A.N. 1947. Children born during the siege of Leningrad in 1942. J. Pediat. 30 : 250-259.

ARDRAN, G.M. and BROWN, T.H. 1964. X-ray diagnosis of pregnancy in sheep with special reference to the determination of the number of foetuses. J. agric. Sci., Camb. 63 : 205-207.

ARMSTRONG, D.G. 1965a. The utilization of rumen digesta. In Beef Production & Marketing (ed. R.Q. Cannell). Br. Grassld. Soc. Occas. Symp. No. 2, pp. 12-20.

ARMSTRONG, D.G. 1965b. Carbohydrate metabolism in ruminants and energy supply. In Physiology of Digestion in the Ruminant (ed. R.W. Dougherty, R.S. Allen, W. Burroughs, N.L. Jacobson and A.D. McGilliard), Butterworth, London.

BARCROFT, J., McANALLY, R.A. and PHILLIPSON, A.T. 1944. The absorption of volatile acids from the alimentary tract of the sheep and other animals. J. exp. Biol. 20 : 120-129.

BARNETT, A.J.G. and REID, R.L. 1961. Reactions in the Rumen. Arnold, London.

BARNICOAT, C.R., LOGAN, A.G. and GRANT, A.I. 1949. Milk secretion studies with New Zealand Romney Ewes. I and II. J. agric. Sci., Camb. 39 : 44-55.

BASSETT, J.M., MILLS, S.C. and REID, R.L. 1966. The influence of cortisol on glucose utilization in sheep. Metabolism 15 : 922-932.

BASSETT, J.M. and WALLACE, A.L.C. 1966. Short-term effects of ovine growth hormone on plasma glucose, free fatty acids and ketones in sheep. Metabolism 15 : 933-944.

BASU, A. 1960. Factors affecting the level of non-esterified fatty acids in blood. Ph.D. Thesis, Fac. Med., Univ. Edinburgh.

BASU, A., PASSMORE, R. and STRONG, J.A. 1960. The effect of exercise on the level of non-esterified fatty acids in the blood. Q. Jl. exp. Physiol. 45 : 312-317.

BENNETT, D., AXELSEN, A. and CHAPMAN, H.W. 1964. The effect of nutritional restriction during early pregnancy on numbers of lambs born. Proc. Aust. Soc. Anim. Prod. 5 : 70-71.

BENZIE, D. 1950. Growth of skeleton of the foetal sheep. Br. vet. J. 106 : 231-234.

BENZIE, D. 1951. X-ray diagnosis of pregnancy in ewes. Br. vet. J. 107 : 3-6.

BERGMAN, E.N. 1961. Glucose turnover rates in pregnant and non-pregnant sheep. Nature, Lond. 202 : 1333.

BERGMAN, E.N., REID, R.S., MURRAY, M.G., BROCKWAY, J.M. and WHITELAW, F.G. 1965. Interconversions and production of volatile fatty acids in the sheep rumen. Biochem. J. 97 : 53-58.

BERGMAN, E.N., ROE, W.E. and KON, K. 1966. Quantitative aspects of propionate metabolism and gluconeogenesis in sheep. Am. J. Physiol. 211 : 793-799.

BERRY, M.N., WILLIAMSON, D.H. and WILSON, M.B. 1965. Concentrations of acetoacetate and D-(-)-3-hydroxybutyrate in rat liver and blood. Biochem. J. 94 : 17C-19C.

BLAXTER, K.L. 1944. Experiments on the use of home grown foods for milk production. II The effect of feeding concentrated and bulky foods prior to calving on subsequent milk production. J. agric. Sci., Camb. 34 : 27-34.

BLAXTER, K.L. 1957. The effects of defective nutrition during pregnancy in farm livestock. Proc. Nutr. Soc. 16 : 52-58.

BLAXTER, K.L. 1961. Energy utilization in the ruminant. In Digestive Physiology and Nutrition of the Ruminant (ed. D. Lewis), Butterworth, London.

BLAXTER, K.L. 1962. The Energy Metabolism of Ruminants. Hutchinson, London.

BLAXTER, K.L. 1965. (ed.) Energy Metabolism. Eur. Ass. Anim. Prod. No. 11. Academic Press, London.

BONSMA, J.C. 1949. Breeding cattle for increased adaptability to tropical and subtropical environments. J. agric. Sci., Camb. 39 : 204-221.

BRAUDE, R. and WALKER, D.M. 1949. Mortality, weight and body measurements at birth of Dairy Shorthorn calves. J. agric. Sci., Camb. 39 : 156-163.

BRODY, S. 1945. Bioenergetics and Growth. Reinhold, New York.

BURTIS, C.A., JACKSON, H.D., PACKETT, L.V. and GOETSCH, D.V.M. 1966. Effects of epinephrine, hydrocortisone and thyroxine on phlorizin-induced ketosis in fasted, nonpregnant ewes. Am. J. vet. Res. 27 : 879-883.

BUTTERFIELD, R.M. 1966. The effect of nutritional stress and recovery on the body composition of cattle. Res. vet. Sci. 7 : 168-179.

CLOETE, J.H.L. 1939. Prenatal growth in the Merino sheep. Onderstepoort J. vet. Sci. Anim. Ind. 13 : 418-558.

COOP, I.E. 1950. The effect of level of nutrition during pregnancy and during lactation on lamb and wool production of grazing sheep. J. agric. Sci., Camb. 40 : 311-340.

COOP, I.E. 1962a. Liveweight-productivity relationships in sheep. I Liveweight and reproduction. N.Z. Jl. agric. Res. 5 : 249-264.

COOP, I.E. 1962b. The energy requirements of sheep for maintenance and gain. I Pen fed sheep. J. agric. Sci., Camb. 58 : 179-186.

COOP, I.E. 1964. Liveweight, flushing and fertility. Sheepfmg A. pp. 122-132.

COOP, I.E. 1967. The efficiency of feed utilization. Proc. N.Z. Soc. Anim. Prod. 27 : 154-165.

COOP, I.E. and HILL, M.K. 1962. The energy requirements of sheep for maintenance and gain. II Grazing sheep. J. agric. Sci., Camb. 58 : 187-199.

DOUGHERTY, R.W., ALLEN, R.S., BURROUGHS, W., JACOBSON, N.L. and McGILLIARD, A.D. 1965. (ed.) Physiology of Digestion in the Ruminant. Butterworth, London.

EADIE, J. 1967. The nutrition of grazing hill sheep; utilization of hill pastures. Fourth report, Hill Farming Research Organisation, Edinburgh, pp. 38-50.

EDINBURGH and EAST of SCOTLAND COLLEGE of AGRICULTURE. 1966. Hill sheep report, 1965-1966.

EVERITT, G.C. 1964. Maternal undernutrition and retarded foetal development in Merino sheep. Nature, Lond. 201 : 1341-1342.

EVERITT, G.C. 1966. Maternal food consumption and foetal growth in Merino sheep. Proc. Aust. Soc. Anim. Prod. 6 : 91-101.

EVERITT, G.C. 1967. Residual effects of prenatal nutrition on the postnatal performance of Merino sheep. Proc. N.Z. Soc. Anim. Prod. 27 : 52-68.

FORBES, T.J. and ROBINSON, J.J. 1966. Winter management of in-lamb ewes with special reference to housing and feeding. Rec. agric. Res. 15 : 1-11.

FORD, E.J.H., CLARK, J.W. and GALLUP, A.L. 1963. The detection of foetal numbers in sheep by means of X-rays. Vet. Rec. 75 : 958-960.

FRASER, A. 1960. Animal Husbandry Heresies. Crosby Lockwood, London.

FRASER, A.H.H. 1939. Economic aspects of the Scottish sheep industry. Trans. R. Highld agric. Soc. Scotl. V : 51 : 39-57.

FREDRICKSON, D.S. and GORDON, R.S. 1958. Transport of fatty acids. Physiol. Rev. 38 : 585-630.

FRITZ, I.B. 1961. Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. Physiol. Rev. 41 : 52-129.

GARDNER, R.W. and HOGUE, D.E. 1963. Studies on the TDN requirements of pregnant and lactating ewes. J. Anim. Sci. 22 : 410-417.

GARRETT, W.N., MEYER, J.H. and LOFGREEN, G.P. 1959. The comparative energy requirements of sheep and cattle for maintenance and gain. J. Anim. Sci. 18 : 528-547.

GILL, J.C. and THOMSON, W. 1954a. Some aspects of the nutrition of the in-lamb ewe. Proc. Br. Soc. Anim. Prod. pp. 35-44.

GILL, J.C. and THOMSON, W. 1954b. The production of ketonaemia and pregnancy toxaemia in ewes through manipulation of the feeding level. J. agric. Sci., Camb. 45 : 229-240.

GOTTSCHALK, L.A., STONE, W.N., GLESER, G.C. and IACONO, J.M. 1966. Anxiety levels in dreams : relation to changes in plasma free fatty acids. Science, Wash., D.C. 153 : 654-657.

GRAHAM, N.McC. 1964. Energy exchanges of pregnant and lactating ewes. Aust. J. agric. Res. 15 : 127-141.

GRAY, F.V., WELLER, R.A., PILGRIM, A.F. and JONES, G.B. 1967. Rates of production of volatile fatty acids in the rumen. V Evaluation of fodders in terms of volatile fatty acid produced in the rumen of the sheep. Aust. J. agric. Res. 18 : 625-634.

GUNN, R.G. 1967. A note on hill ewe mortality. Anim. Prod. 9 : 263-264.

GUYER, P.Q. and DYER, A.J. 1954. Study of factors affecting sheep production. Missouri agric. Exp. Stn., Bull. No. 558.

HARTMANN, P.E. and LASCELLES, A.K. 1965. The effect of starvation on the uptake of the precursors of milk fat by the bovine mammary gland. Aust. J. biol. Sci. 18 : 1025-1034.

HIGHT, G.K. and BARTON, R.A. 1965. The effects of undernutrition and re-alimentation on the Romney ewe. J. agric. Sci., Camb. 64 : 413-424.

HILL FARMING RESEARCH ORGANISATION. 1958. First report, Hill Farming Research Organisation, Edinburgh.

HODGE, R.W. 1966. The effect of nutritional restriction during early and mid pregnancy on the reproduction performance of crossbred ewes. Aust. J. exp. Agric. Anim. Husb. 6 : 311-313.

HUGGETT, A.St.G. and NIXON, D.A. 1957. Use of glucose oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. Lancet, 273 : 368-370.

HUNGATE, R.E., PHILLIPS, G.D., McGREGOR, A., HUNGATE, D.P. and BUECHNER, H.K. 1959. Microbial fermentation in certain mammals. Science, Wash., D.C. 130 : 1192-1194.

HYTTEN, F.E. 1963. Nutritional aspects of foetal growth. Proc. 6th Int. Cong. Nutr. pp. 59-65.

HYTTEN, F.E. and LEITCH, I. 1964. The Physiology of Human Pregnancy. Blackwell, Oxford.

ISSEKUTZ, B. jr, BORTZ, W.M., MILLER, H.I. and WROLDSEN, A. 1967. Plasma free fatty acid response to exercise in obese humans. Metabolism 16 : 492-502.

JACKSON, H.D., BURTIS, C.A. and GOETSCH, D.V.M. 1966. Effects of phlorizin and heparin on ketonaemia in fasted, non-pregnant ewes. Am. J. vet. Res. 27 : 885-890.

JEANRENAUD, B. 1961. Dynamic aspects of adipose tissue metabolism: a review. Metabolism 10 : 535-581.

JEFFERIES, B.C. 1961. Body condition scoring and its use in management. Tasm. J. Agric. 32 : 19-21.

JEFFERIES, B.C. and FEARN, J.T. 1956. Over-fat ewes have light lambs. J. Dep. Agric. S. Aust. 60 : 133-134.

KIRTON, A.H. and BARTON, R.A. 1958a. Live weight loss and its components in Romney ewes subjected to l-thyroxine therapy and a low plane of nutrition. I Effects on live weight, carcass weight and carcass composition. J. agric. Sci., Camb. 51 : 265-281.

KIRTON, A.H. and BARTON, R.A. 1958b. Live weight loss and its components in Romney ewes subjected to l-thyroxine therapy and a low plane of nutrition. II Effects on some non-carcass components of live weight. J. agric. Sci., Camb. 51 : 282-288.

KNOBIL, E. and HOTCHKISS, J. 1964. Growth hormone. A. Rev. Physiol. 26 : 47-74.

KREBS, H.A. 1965. Some aspects of gluconeogenesis (the relations between gluconeogenesis and ketogenesis). In Energy Metabolism (ed. K.L. Blaxter), Eur. Ass. Anim. Prod. No. 11. Academic Press, London.

KREBS, H.A. 1966. Bovine ketosis. Vet. Rec. 78 : 187-192.

KRONFELD, D.S. 1958. The fetal drain of hexose in ovine pregnancy toxaemia. Cornell Vet. 48 : 394-404.

LAMBOURNE, L.J. and REARDON, T.F. 1963. Effect of environment on the maintenance requirement of Merino wethers. Aust. J. agric. Res. 14 : 272-293.

LANDS, W.E.M. 1965. Lipid metabolism. A. Rev. Biochem. 34 : 313-346.

LANGLANDS, J.P., CORBETT, J.L., McDONALD, I. and PULLAR, J.D. 1963a. Estimates of the energy required for maintenance by adult sheep. I Housed sheep. Anim. Prod. 5 : 1-9.

LANGLANDS, J.P., CORBETT, J.L., McDONALD, I. and PULLAR, J.D. 1963b. Estimates of the energy required for maintenance by adult sheep. II Grazing sheep. Anim. Prod. 5 : 11-16.

LENG, R.A. and BRETT, D.J. 1966. Simultaneous measurements of the rates of production of acetic, propionic and butyric acids in the rumen of sheep on different diets and the correlation between production rates and concentrations of these acids in the rumen. Br. J. Nutr. 20 : 541-552.

LENG, R.A., STEEL, J.W. and LUICK, J.R. 1967. Contribution of propionate to glucose synthesis in sheep. Biochem. J. 103 : 785-790.

LEWIS, D. 1961. (ed.) Digestive Physiology and Nutrition of the Ruminant. Butterworth, London.

LINDSAY, D.B. 1959a. The significance of carbohydrate in ruminant metabolism. Vet. Revs. Annot. 5 : 103-128.

LINDSAY, D.B. 1959b. Non-esterified fatty acids in sheep plasma. Biochem. J. 73 : 10P.

LINDSAY, D.B. 1961. Endocrine control of metabolism in ruminants. In Digestive Physiology and Nutrition of the Ruminant (ed. D. Lewis), Butterworth, London.

MANNS, J.G. and BODA, J.M. 1965. Effects of ovine growth hormone and prolactin on blood glucose, serum insulin, plasma non-esterified fatty acids and amino nitrogen in sheep. Endocrinology 76 : 1109-1114.

MANNS, J.G. and BODA, J.M. 1967. Insulin release by acetate, propionate, butyrate, and glucose in lambs and adult sheep. Am. J. Physiol. 212 : 747-755.

MANNS, J.G., BODA, J.M. and WILLES, R.F. 1967. Probable role of propionate and butyrate in control of insulin secretion in sheep. Am. J. Physiol. 212 : 756-764.

MASORO, E.J. 1966. Effect of cold on metabolic use of lipids. Physiol. Rev. 46 : 67-101.

MENAHAN, L.A., SCHULTZ, L.H. and HOEKSTRA, W.G. 1966a. Factors affecting ketogenesis from butyric acid in the ruminant. J. Dairy Sci. 49 : 835-845.

MENAHAN, L.A., SCHULTZ, L.H. and HOEKSTRA, W.G. 1966b. Relationship of ketone body metabolism and carbohydrate utilization to fat mobilization in the ruminant. J. Dairy Sci. 49 : 957-961.

McANALLY, R.A. and PHILLIPSON, A.T. 1942. Absorption of volatile acids from the rumen of the sheep. J. Physiol. 101 : 13P-14P.

McCLYMONT, G.L. and LAMBOURNE, J. 1958. Interactions between planes of nutrition during early and late pregnancy. Proc. Aust. Soc. Anim. Prod. 2 : 135-138.

NATIONAL RESEARCH COUNCIL. 1957. Nutrient Requirements of Domestic Animals. No. 5 Nutrient Requirements of Sheep. Publs natn. Res. Coun., Wash., no. 504.

NATIONAL RESEARCH COUNCIL. 1964. Nutrient Requirements of Domestic Animals. No. 5 Nutrient Requirements of Sheep. Publs natn. Res. Coun., Wash., no. 1193.

OLSON, J.A. 1966. Lipid metabolism. A. Rev. Biochem. 35 : 559-598.

PALSSON, H. 1955. Conformation and body composition. In Physiology of Farm Animals (ed. J. Hammond), Butterworth, London.

PALSSON, H. and VERGES, J.B. 1952. Effects of the plane of nutrition on growth and the development of carcass quality in lambs. I The effects of high and low planes of nutrition at different ages. J. agric. Sci., Camb. 42 : 1-92.

PANARETTO, B.A. 1964. Body composition in vivo. VI The composition of ewes during prolonged undernutrition. Aust. J. agric. Res. 15 : 771-787.

PAPADOPOULOS, J.C. and ROBINSON, T.J. 1957. Fat lamb studies in Victoria. II The interaction between pre- and postnatal planes of nutrition on the production of wool and lambs by crossbred sheep. Aust. J. agric. Res. 8 : 471-493.

PATTERSON, D.S.P. 1963. Some observations on the estimation of non-esterified fatty acid concentrations in cow and sheep plasma. Res. vet. Sci. 4 : 230-237.

PATTERSON, D.S.P. 1964. The effect of intravenous glucose on depot fat mobilization in the sheep. Res. vet. Sci. 5 : 286-293.

PEART, J.N. 1967. The effect of different levels of nutrition during late pregnancy on the subsequent milk production of Blackface ewes and on the growth of their lambs. J. agric. Sci., Camb. 68 : 365-371.

RADLOFF, H.D. and SCHULTZ, L.H. 1966. Hormonal regulation of plasma free fatty acid concentration in ruminants. J. Dairy Sci. 49 : 971-975.

RADLOFF, H.D., SCHULTZ, L.H. and HOEKSTRA, W.G. 1966. Relationship of plasma free fatty acids to other blood components in ruminants under various physiological conditions. J. Dairy Sci. 49 : 179-182.

REID, R.L. 1950. Studies on the carbohydrate metabolism of sheep. II The uptake by the tissues of glucose and acetic acid from the peripheral circulation. Aust. J. agric. Res. 1 : 338-354.

REID, R.L. 1958. Husbandry of the pregnant ewe. Wool Technol. Sheep Breed. 5 : 91-95.

REID, R.L. 1960. The determination of ketone bodies in blood. Analyst, Lond. 85 : 265-271.

REID, R.L. 1962. Studies on the carbohydrate metabolism of sheep. XVI Partition of ketone bodies in blood, tissue and urine. Aust. J. agric. Res. 13 : 307-319.

REID, R.L. 1963. The nutritional physiology of the pregnant ewe. J. Aust. Inst. agric. Sci. 29 : 215-223.

REID, R.L. 1968. The physio-pathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxaemia. In press.

REID, R.L. and HINKS, N.T. 1962a. Studies on the carbohydrate metabolism of sheep. XVII Feed requirements and voluntary feed intake in late pregnancy, with particular reference to prevention of hypoglycaemia and hyperketonaemia. Aust. J. agric. Res. 13 : 1092-1111.

REID, R.L. and HINKS, N.T. 1962b. Studies on the carbohydrate metabolism of sheep. XVIII The metabolism of glucose, free fatty acids, ketones and amino acids in late pregnancy and lactation. Aust. J. agric. Res. 13 : 1112-1123.

REID, R.L. and HINKS, N.T. 1962c. Studies on the carbohydrate metabolism of sheep. XIX The metabolism of glucose, free fatty acids, and ketones after feeding and during fasting or undernourishment of non-pregnant, pregnant, and lactating ewes. Aust. J. agric. Res. 13 : 1124-1136.

REID, R.L. and HOGAN, J.P. 1959. Studies on the carbohydrate metabolism of sheep. VIII Hypoglycaemia and hyperketonaemia in undernourished and fasted pregnant ewes. Aust. J. agric. Res. 10 : 81-96.

RINEY, T. 1955. Evaluating condition of free-ranging red deer (Cervus claphus), with special reference to New Zealand. N.Z. Jl. Sci. Technol. 36B : 429-463.

ROBINSON, J.F., CURRIE, D.C. and PEART, J.N. 1961. Feeding hill ewes. Trans. R. Highld. agric. Soc. Scotl. VI : 6 : 31-46.

ROBINSON, P. 1948. The effect of supermaintenance and submaintenance diets on mature Border Leicester-Cheviot ewes. J. agric. Sci., Camb. 38 : 345-350.

ROE, W.E., BERGMAN, E.N. and KON, K. 1966. Absorption of ketone bodies and other metabolites via the portal blood of sheep. Am. J. vet. Res. 27 : 729-736.

RUDMAN, D. 1963. The adipokinetic action of polypeptide and amine hormones upon the adipose tissue of various animal species. J. Lipid. Res. 4 : 119-129.

RUSSEL, A.J.F., GUNN, R.G., SKEDD, E. and DONEY, J.M. 1968. Relationships between chemical and physical composition of Scottish Blackface ewes. Anim. Prod. 10 : 53-58.

SCARISBRICK, R. and PUGH, P.D.S. 1957. The passage of acetate across the placenta and its uptake by the foetal sheep. Br. vet. J. 113 : 328-341.

SCHINCKEL, P.G. 1963. Nutrition and sheep production : a review. Proc. Wld Conf. Anim. Prod. 1 : 199-248.

SCHINCKEL, P.G. and SHORT, B.F. 1961. The influence of nutritional level during pre-natal and early post-natal life on adult fleece and body characters. Aust. J. agric. Res. 12 : 176-202.

SHAPIRO, B. 1967. Lipid metabolism. Am. Rev. Biochem. 36 : 247-270.

SMITH, C.A. 1947. Effects of maternal undernutrition upon the newborn infant in Holland (1944-1945). J. Pediat. 30 : 229-243.

SMOLIAK, S. and SLEN, S.B. 1958. Effects of certain supplements and methods of feeding during late pregnancy on lamb and wool production of mature ewes. Can. J. Anim. Sci. 38 : 91-96.

SNEDECOR, G.W. 1957. Statistical Methods. Iowa State College Press, Ames, Iowa.

SOMOGYI, M. 1952. Notes on sugar determination. J. biol. Chem. 195 : 19-23.

SOSKIN, S. and LEVINE, R. 1952. Carbohydrate Metabolism. Univ. Chicago Press, Chicago, Illinois.

SUTHERLAND, T.M. 1963. The metabolism of short chain fatty acids in the ruminant. In Progress in Nutrition and Allied Sciences (ed. D.P. Cuthbertson), Oliver and Boyd, Edinburgh.

SWIFT, R.W. 1957. The calorific value of TDN. J. Anim. Sci. 16 : 753-756.

TAPLIN, D.E. and EVERITT, G.C. 1964. The influence of prenatal nutrition on postnatal performance of Merino lambs. Proc. Aust. Soc. Anim. Prod. 5 : 72-81.

THOMPSON, D.S. 1950. The effect of nutrition on the in-lamb ewe. J. Dep. Agric. S. Aust. 53 : 352-355.

THOMSON, A.M. and THOMSON, W. 1949. Lambing in relation to the diet of the pregnant ewe. Br. J. Nutr. 2 : 290-305.

THOMSON, W. and FRASER, A.H.H. 1939. Feeding concentrates to the in-lamb ewe. The effects in lambing results. Scott. J. Agric. 22 : 71-73.

THOMSON, W. and THOMSON, A.M. 1953. Effect of diet on milk yield of the ewe and growth of her lamb. Br. J. Nutr. 7 : 263-274.

THOMSON, W.A. and AITKEN, F.C. 1959. Diet in relation to reproduction and viability of the young. Part II. Sheep : World survey of reproduction and review of feeding experiments. Commonw. Agric. Bur., England.

TOVE, S.B. 1965. Fat metabolism in ruminants. In Physiology of Digestion in the Ruminant (ed. R.W. Dougherty, R.S. Allen, W. Burroughs, N.L. Jacobson and A.D. McGilliard), Butterworth, London.

ULYATT, M.J. 1967. The use of a steady state feeding system in nutrition experiments with ruminants. Proc. N.Z. Soc. Anim. Prod. 27 : 181-192.

ULYATT, M.J. and BARTON, R.A. 1963. A comparison of the chemical and dissectible carcass composition of New Zealand Romney Marsh ewes. J. agric. Sci., Camb. 60 : 285-289.

UNDERWOOD, E.J., SHIER, F.L. and CARISS, H.G. 1943. Studies in sheep husbandry in Western Australia. IV The influence of a high level of prenatal feeding in lamb production. J. Dep. Agric. West. Aust. 20 : 288-297.

VERGES, J.B. 1939. The effect of plane of nutrition of the ewe on weight and development of the lamb at birth. Proc. 4th Int. Congr. Anim. Breed. p. 83.

WALLACE, L.R. 1948a. The growth of lambs before and after birth in relation to the level of nutrition. Part I. J. agric. Sci., Camb. 38 : 93-153.

WALLACE, L.R. 1948b. The growth of lambs before and after birth in relation to the level of nutrition. Part II. J. agric. Sci., Camb. 38 : 243-302.

WALLACE, L.R. 1961. Influence of live weight and condition on ewe fertility. Proc. Ruakura Fmrs' Conf. Week, pp. 14-25.

WELLER, R.A., GRAY, F.V., PILGRIM, A.F. and JONES, G.B. 1967. The rates of production of volatile fatty acids in the rumen. IV Individual and total volatile fatty acids. Aust. J. agric. Res. 18 : 107-118.

WEST, C.E. and ANNISON, E.F. 1964. Metabolism of palmitate in sheep. Biochem. J. 92 : 573-578.

WHITE, A., HANDLER, P. and SMITH, E.L. 1964. Principles of Biochemistry. McGraw-Hill, London.

WHITING, F. and SLEN, S.B. 1958. The influence of protein and energy content of the ration on lamb and wool production of range ewes. Can. J. Anim. Sci. 38 : 208-216.

WILLIAMS, W.F., LEE, S.D., HEAD, H.H. and LYNCH, J. 1963. Growth hormone effects on bovine blood plasma fatty acid concentration and metabolism. J. Dairy Sci. 46 : 1405-1408.

WILLIAMS, W.F., WEISSHAAR, A.G. and LAUTERBACH, G.E. 1966. Lactogenic hormone effects on plasma non-esterified fatty acids and blood glucose concentrations. J. Dairy Sci. 49 : 106-107.

WINTERS, L.M. and FEUFFEL, G. 1936. Studies on the physiology of reproduction in the sheep. IV Foetal development. Tech. Bull. Minn. agric. Exp. Stn. No. 118.

WRIGHT, P.L., POPE, A.L. and PHILLIPS, P.H. 1962. Effect of protein and energy intake on lamb production and certain blood constituents of ewes. J. Anim. Sci. 21 : 602-610.

IX - APPENDICES

APPENDIX 1

Live weights and weights of body components recorded at slaughter (kg) (cf. Tables 3 and 4) (figures corrected to first decimal place)

Sheep No	Initial live weight	Live weight at slaughter	Carcass	Omental and Mesenteric fatty tissues	Liver	Heart	Blood	Remainder*
				GROUP I				
239	43.5	40.5	15.2	0.5	0.5	0.2	1.3	9.0
242	50.0	46.8	20.0	1.1	0.7	0.3	1.7	10.5
249	48.0	45.0	19.6	1.6	0.7	0.3	1.7	11.1
255	56.5	51.4	23.2	1.2	0.8	0.3	2.5	11.9
256	52.0	46.4	20.5	1.1	0.8	0.3	2.0	10.9
272	56.0	50.5	19.6	1.2	0.8	0.3	2.1	11.3
				GROUP II				
241	49.5	36.4	14.3	0.8	0.5	0.2	1.2	7.9
248	50.3	42.7	16.1	0.8	0.6	0.2	1.8	9.1
252	48.5	43.6	17.2	1.2	0.5	0.3	1.9	9.0
261	50.5	41.4	16.6	1.6	0.6	0.3	1.8	9.6
265	40.0	34.6	13.9	0.4	0.5	0.2	1.7	8.4
267	50.5	38.2	15.2	1.3	0.6	0.3	1.5	9.0
				GROUP III				
232	44.0	38.4	12.2	0.3	0.5	0.2	1.6	8.3
233	51.0	44.4	16.8	1.4	0.6	0.3	2.1	8.7
236	44.5	37.8	13.3	0.5	0.5	0.3	1.5	8.2
245	54.0	40.1	14.4	0.5	0.6	0.3	1.8	9.2
247	53.5	45.7	15.4	0.5	0.6	0.2	2.1	9.8
254	41.5	32.5	11.5	0.1	0.5	0.3	1.6	7.4
259	47.5	41.5	13.4	0.6	0.6	0.3	1.8	8.5
262	55.0	45.6	15.9	1.2	0.6	0.3	1.8	9.4
264	57.5	48.5	16.8	0.7	0.7	0.3	2.2	9.9
274	62.0	50.6	16.8	1.0	0.6	0.3	2.3	9.1
277	49.0	42.7	15.9	1.0	0.5	0.2	1.9	8.8

* 'Remainder' includes head, skin, metatarsals, metacarpals and all internal organs and parts not listed separately, but excluding uterine contents.

APPENDIX 2

Frozen carcass weights and weights of body components recorded at dissection (kg) (cf. Tables 3 and 4) (figures corrected to first decimal place)

Sheep No	Frozen carcass weight	Left half carcass	Muscular tissue and fatty tissue	Subcutaneous fatty tissue	Perirenal fatty tissue	Bone	Pericardial fatty tissue
			GROUP I				
239	13.3	6.4	4.7	0.2	0.2	1.2	0.1
242	18.3	9.8	6.9	0.6	0.4	1.7	0.1
249	17.9	8.9	5.9	1.0	0.5	1.2	0.1
255	21.2	10.7	7.4	0.8	0.3	1.7	0.1
256	18.6	9.2	6.5	0.8	0.4	1.4	0.1
272	17.5	8.6	6.1	0.6	0.3	1.5	0.1
			GROUP II				
241	12.3	6.2	4.5	0.2	0.1	1.3	0.1
248	14.1	7.0	5.1	0.2	0.1	1.4	0.1
252	15.7	8.0	5.9	0.3	0.3	1.4	0.1
261	14.8	7.4	5.2	0.5	0.3	1.3	0.1
265	12.0	6.1	4.6	0.1	0.1	1.3	-
267	13.4	6.7	4.8	0.2	0.2	1.3	0.1
			GROUP III				
232	10.4	5.0	3.7	-	-	1.1	-
233	15.6	7.9	5.5	0.5	0.3	1.5	0.1
236	11.4	5.7	4.2	-	0.1	1.1	0.1
245	12.5	6.3	4.6	0.1	0.1	1.4	0.1
247	13.4	6.8	5.2	-	-	1.4	-
254	9.8	4.9	3.4	0.1	0.1	1.1	0.1
259	11.4	5.7	4.2	-	0.1	1.3	-
262	14.3	7.2	5.2	0.2	0.3	1.3	0.1
264	14.2	7.1	5.1	0.1	0.1	1.7	0.1
274	15.1	7.6	5.7	0.1	0.3	1.4	0.1
277	14.6	7.7	5.7	0.2	0.2	1.5	0.1

APPENDIX 3

Percentages of water and chemical fat in maternal empty body components (cf. Tables 3 and 4) (figures corrected to first decimal place)

Sheep No	Muscular and fatty tissues		Subcutaneous fatty tissue		Omental and Mesenteric fatty tissues		Perirenal fatty tissues	
	Water	Fat	Water	Fat	Water	Fat	Water	Fat
				GROUP I				
239	67.4	11.1	22.6	56.2	24.7	73.0	18.3	78.4
242	63.6	17.0	7.3	80.5	15.7	81.1	9.5	86.6
249	63.9	16.2	7.1	84.6	15.6	78.4	8.3	82.4
255	65.5	13.0	10.2	71.1	17.8	80.3	12.3	77.5
256	65.0	13.7	11.7	80.5	16.3	81.0	14.2	79.8
272	63.4	16.8	7.3	82.0	19.5	76.6	9.0	87.8
				GROUP II				
241	70.2	10.5	32.0	50.9	43.5	51.9	26.3	68.6
248	68.2	10.3	15.1	59.8	39.6	55.9	33.7	63.1
252	65.5	15.1	8.9	79.1	23.9	73.6	13.6	86.8
261	65.4	19.1	8.9	81.2	31.8	68.3	16.5	79.6
265	69.6	7.5	26.6	29.7	50.9	42.0	25.5	68.7
267	68.2	14.0	15.1	69.8	30.0	66.7	16.7	75.8
				GROUP III				
232	76.3	4.0	31.8	8.1	83.5	7.8	72.8	15.6
233	65.7	15.6	18.5	72.3	20.7	76.1	18.1	76.6
236	73.8	6.6	28.6	31.7	66.7	27.2	60.5	29.7
245	71.6	10.0	33.8	34.6	59.8	34.3	47.5	45.3
247	71.8	6.0	34.5	30.3	57.5	31.8	49.8	36.2
254	77.0	5.8	54.9	11.7	69.0	24.8	67.8	23.8
259	72.2	7.1	56.7	23.3	60.7	33.2	59.7	31.1
262	66.7	14.2	24.3	62.7	29.7	59.1	27.9	68.8
264	71.4	7.7	32.2	42.5	60.7	32.7	51.3	36.7
274	69.5	11.5	32.3	57.1	38.9	56.1	33.3	62.4
277	67.2	15.0	12.4	75.4	20.7	75.3	17.8	76.7

APPENDIX 3

Percentages of water and chemical fat in maternal empty body components (cf. Tables 3 and 4) (figures corrected to first decimal place)

Bone		Liver		Pericardial fatty tissue		Remainder	
Water	Fat	Water	Fat	Water	Fat	Water	Fat
			GROUP I				
28.7	36.9	71.4	3.5	32.0	62.4	69.5	8.6
27.5	26.8	72.0	1.9	17.7	79.6	67.4	10.6
25.6	24.3	71.8	3.1	21.0	76.4	68.8	9.2
26.4	22.6	70.5	3.9	23.8	71.9	67.6	8.5
25.8	28.3	71.5	4.2	23.5	73.1	67.3	10.0
29.8	29.5	71.7	4.6	36.5	57.5	69.1	9.3
			GROUP II				
33.5	28.6	72.7	3.6	29.5	64.9	69.3	9.5
28.3	31.1	73.4	3.9	33.7	63.1	70.4	8.0
26.8	30.0	70.6	4.3	29.1	68.1	70.2	9.9
27.7	34.2	71.7	9.4	30.7	70.1	69.2	9.7
25.3	21.1	70.3	3.9	29.8	64.3	72.2	6.0
31.2	27.5	69.8	7.5	30.5	69.5	69.7	10.5
			GROUP III				
44.2	19.3	72.2	7.8	52.3	41.3	73.5	5.5
45.6	23.3	71.5	5.2	26.9	71.4	67.1	11.4
42.3	20.7	69.4	5.6	46.4	51.5	70.0	8.7
33.5	26.4	70.1	6.0	35.6	52.4	71.6	8.5
36.1	16.4	69.2	4.9	51.3	38.2	68.2	5.6
39.3	26.6	70.9	6.9	58.2	35.6	70.5	7.0
39.1	12.0	70.2	5.2	39.2	55.1	71.7	7.6
35.0	25.9	74.9	5.7	15.8	60.6	53.5	10.0
38.4	27.6	69.6	3.9	36.9	56.1	68.8	8.7
31.8	23.8	69.6	6.3	32.9	67.3	65.7	11.6
32.8	25.4	68.9	6.9	23.0	70.2	67.2	11.0

APPENDIX 4

Plasma FFA concentrations (μeq/l) in ewes in first Lephinmore field investigation (cf. Figure 5)

Sheep No	10.3.64	17.3.64	31.3.64	7.4.64	14.4.64	21.4.64	28.4.64	5.5.64
Maiden ewes : single lambs								
954	521	685	698	564	763	617	652	1202
964	1298	629	1350	712	917	492	609	645
965	1056	593	1310	1136	1158	750	1343	1248
980	1386	1146	870	523	809	788	1069	1099
992	1247	621	1294	782	1801	721	828	1161
Mature ewes : single lambs								
379	521	705	738	543	876	425	-	-
386	851	269	614	490	871	492	-	-
472	400	433	998	963	668	642	661	550
482	363	493	1294	942	992	-	-	-
494	688	248	590	658	813	338	425	-
499	395	361	502	473	631	454	567	-
505	553	501	610	597	564	-	-	-
506	753	277	638	819	730	-	-	-
792	595	321	646	523	950	250	283	-
809	963	1038	690	856	983	854	605	-
824	707	1419	1646	823	925	871	-	-
Mature ewes : twin lambs								
511	819	609	1014	712	822	1046	-	-
513	460	633	886	1621	1324	462	-	-
Non-pregnant ewes								
490	437	577	526		577	367	313	289
807	963	1038	690	856	983	854	605	302
998	967	946	594	333	614	362	597	529

APPENDIX 5

Plasma FFA and ketone concentrations and lamb birth-weights from second Lephinmore field investigation (cf. Figures 6 and 7)

Sheep No	23.2.65		23.3.65		30.3.65		13.4.65		Lamb birth-weight (kg)
	FFA (μeq/l)	Ketones (mg %)	FFA (μeq/l)	Ketones (mg %)	FFA (μeq/l)	Ketones (mg %)	FFA (μeq/l)	Ketones (mg %)	
Twin-bearing ewes									
601	813	2.2	936	5.7	1073	7.4			6.3
632	575	1.3	618	2.7	1182	3.7	1319	5.4	5.4
640	1753	2.6	818	4.9	1109	8.5	1531	12.0	7.6
642	740	1.2	836	4.5	1091	7.0	1336	12.1	6.8
646	566	1.4	1164	4.8	1082	7.8	1133	7.5	6.2
Single-bearing ewes									
603	420	1.6	491	1.9	445	3.0	735	6.5	3.3
605	721	1.8	336	2.4	727	3.3	681	5.3	4.5
608	977	1.6	800	2.1	1018	3.8	850	2.7	3.9
610	438	1.6	436	2.1	1018	3.2			4.2
611	721	2.1			918	7.4	1195	6.1	4.1
612	466	1.6			1309	3.8	1177	2.8	3.9
613	1635	1.8	627	3.3	1327	5.7	708	2.4	2.7
623	1059	2.2			1336	6.5	1513	3.8	4.1
633	703	0.9	700	3.7	827	5.3	956	7.3	4.5
636	457	2.9	573	2.4	573	3.3	717	3.1	4.2
637	813	2.2	1309	4.1	1327	8.9	991	8.4	4.4
643	420	2.1			1118	3.8	1876	5.0	4.0
644	530	1.9			1173	7.6	1204	3.1	4.5
Non-pregnant ewes									
614	676	1.3	491	2.0	582	2.8	628	2.3	
631	877	1.5	691	3.0	1018	3.5	637	1.8	

APPENDIX 6

Plasma FFA concentrations and lamb birth-weights in Sourhope experiment (cf. Figure 8)

Sheep No	Plasma FFA (μeq/l)	Birth-weight (kg)
4	2124	6.2
8	798	3.3
11	815	3.8
14	1283	3.7
16	1819	3.5
19	1524	4.2
20	2266	4.3
25	1681	3.9
27	2686	5.8
28	3071	5.9
32	1246	3.8
900	2116	3.5
901	1687	4.3
904	1185	4.1
906	1430	4.4
907	720	2.8
911	1254	4.6
914	1395	4.6
918	1283	3.4
921	1167	4.0
923	1077	4.2
924	2013	4.1
925	1219	4.0

APPENDIX 7

Plasma parameters and lamb birth-weights from Castlelaw investigation (cf. Figure 9)

Sheep No	FFA (μeq/l)	Ketones (mg %)	Glucose (mg %)	Birth-weight (kg)
101	418	2.6	65	4.3
103	440	8.9	32	3.2
106	582	0.9	64	4.5
201	1181	6.8	32	5.7
202	795	2.3	58	2.7
208	984	4.3	51	3.9
209	851	3.7	37	3.6
215	847	2.4	61	3.2
216	913	2.7	45	4.8
219	705	3.5	39	5.7
227	891	7.7	44	7.3
307	883	5.3	44	4.5
312	1206	2.4	42	3.9
313	496	3.6	58	3.6
316	475	2.2	51	4.1
319	1681	7.8	46	4.5
323	511	4.5	38	5.4
325	986	2.7	42	3.6
328	1181	9.2	33	5.4
329	956	4.7	37	5.7
336	903	2.8	44	4.8
337	1226	2.9	42	6.1
344	636	3.5	43	5.9
347	1206	6.1	29	8.6
348	861	4.8	40	5.0
404	976	2.2	50	4.3
409	1321	2.5	39	6.8

APPENDIX 7 (continued)

Sheep No	FFA (μeq/l)	Ketones (mg %)	Glucose (mg %)	Birth-weight (kg)
412	679	2.3	45	4.5
420	562	4.4	39	4.8
430	1402	2.6	46	4.5
432	1248	3.5	34	4.8
443	606	1.8	47	5.0
444	971	1.9	37	4.8
446	1103	3.9	40	5.4
447	1482	3.6	46	4.5
502	363	2.0	39	2.7
506	703	3.1	37	3.2
507	555	4.9	41	4.8
512	656	1.9	54	3.2
523	820	3.1	56	4.3
530	918	4.4	45	4.5
538	1091	3.5	47	3.6
540	511	1.8	46	4.8
541	1263	2.9	39	4.1
544	962	4.2	35	4.5
560	650	2.8	40	3.9
568	1542	4.4	37	5.7
573	876	2.0	50	3.4
574	1278	4.9	59	5.7
575	1091	3.9	43	6.1
577	730	2.5	45	4.5
581	730	1.9	46	3.2
587	787	2.4	45	5.4
591	1153	2.0	48	4.5

APPENDIX 8

Plasma FFA and ketone concentrations and lamb birth-weights from Glensaugh ewes (cf. p.59 and Figure 10)

Sheep No	6 weeks prepartum		4 weeks prepartum		2 weeks prepartum		1 week prepartum		Birth-weight (kg)
	FFA (μeq/l)	Ketones (mg %)	FFA (μeq/l)	Ketones (mg %)	FFA (μeq/l)	Ketones (mg %)	FFA (μeq/l)	Ketones (mg %)	
20	937	4.7	738	2.8	938	3.5	1036	3.7	8.0
26	698	3.7	722	2.9	1250	5.2	1429	3.9	8.6
30	897	7.5	579	5.4	884	5.7	1277	5.5	8.3
32	778	4.9	516	2.6	705	4.0	1071	2.1	7.3
34	627	3.8	603	2.5	857	2.3	777	3.0	6.5
37	810	3.8	278	1.4	429	2.1	830	1.2	4.6
100	532	3.0	452	1.3	500	1.5	304	2.4	3.5
120	1167	7.5	373	5.0	893	4.7	875	4.0	8.6
127	770	3.7	444	1.3	696	1.2	705	1.7	4.7
136	429	2.6	429	2.4	-	2.9	509	2.1	5.1
139	905	4.8	627	1.4	786	3.7	589	4.2	7.3
148	635	2.3	643	1.7	946	5.0	1402	4.1	8.6
150	1016	3.9	691	4.0	866	3.9	1357	3.6	8.0
152	945	4.6	635	3.4	1063	10.7	1295	4.1	9.4
161	1159	4.8	603	1.8	1250	3.3	1259	5.3	8.6
164	587	3.1	500	1.1	527	1.6	509	1.4	5.3

APPENDIX 9

Phloridzin experiment : Plasma parameters and urinary glucose excretions

Day	Feed intake (g DOM/kg)	Glucose excretion (mg/kg)	Plasma glucose (mg %)	Plasma ketones (mg %)	Plasma FFA (µeq/l)
		Sheep No 67 (cf. Figure 11)			
1*	10	0	65	2.6	530
2*	10	848	56	3.9	704
3*	0	900	58	5.2	809
4*	0	499	38	6.8	2052
5*	0	391	37	11.3	2148
6*	0	416	37	15.7	1817
7*	0	472	36	15.8	1870
8*	0	375	35	18.4	1913
9	10	433	26	24.4	1748
10	10	330	62	16.7	1400
11	10	614	80	2.8	617
		Sheep No 79 (cf. Figure 11)			
1*	10	0	64	3.5	513
2*	10	655	53	4.3	670
3*	0	799	53	5.8	826
4*	0	555	29	12.4	2113
5*	0	306	39	10.3	1757
6*	0	398	34	15.4	1783
7*	0	317	28	17.2	1748
8*	0	310	25	18.0	1826
9	10	295	27	26.7	2000
10	10	449	55	4.5	1035
11	10	322	66	3.2	157

*5 mg phloridzin per kg live weight administered on these days

APPENDIX 9 (continued)

Day	Feed intake (g DOM/kg)	Glucose excretion (mg/kg)	Plasma glucose (mg %)	Plasma ketones (mg %)	Plasma FFA (μeq/l)
		Sheep No 93 (cf. Figure 11)			
1*	10	0	60	2.0	374
2*	10	814	55	5.1	661
3*	0	919	60	4.3	470
4*	0	363	45	4.5	1087
5*	0	55	50	5.0	1417
6*	0	375	34	11.0	1748
7*	0	277	37	14.4	1791
8*	0	237	45	13.4	1652
9	10	509	45	11.7	1713
10	10	42	74	1.9	435
11	10	200	71	1.7	113
		Sheep No 97 (cf. Figure 11)			
1*	10	0	56	1.9	374
2*	10	732	48	3.6	696
3*	0	793	52	5.0	687
4*	0	200	34	9.7	2096
5*	0	428	42	8.0	2504
6*	0	380	36	10.1	2357
7*	0	611	32	14.2	1817
8*	0	225	28	15.6	1661
9	10	293	26	24.3	2000
10	10		58	5.4	487
11	10	257	58	1.9	183

*5 mg phloridzin per kg live weight administered on these days

APPENDIX 10

Means and associated standard errors of plasma parameters, Glensaugh experiment (cf. Figure 12)

Days prepartum	Plasma glucose (mg %)	Plasma ketones (mg %)	Plasma FFA (μeq/l)
	GROUP I		
5			497 ± 45
9	56.1 ± 1.7	2.0 ± 0.2	
12			496 ± 55
19			457 ± 57
23	47.0 ± 1.7	1.7 ± 0.2	
26			399 ± 35
33			387 ± 34
37	43.7 ± 1.4	1.5 ± 0.2	
40			327 ± 30
47			367 ± 34
51	44.3 ± 1.5	1.4 ± 0.1	
54			315 ± 51
	GROUP II		
5	49.0 ± 1.7	3.7 ± 0.4	726 ± 63
12	42.9 ± 1.5	3.9 ± 0.3	864 ± 54
19	41.3 ± 1.7	3.7 ± 0.3	796 ± 41
26	42.1 ± 1.6	3.5 ± 0.4	823 ± 54
33	41.0 ± 1.5	2.7 ± 0.3	663 ± 50
40	42.5 ± 1.3	2.5 ± 0.1	671 ± 40
47	42.9 ± 1.4	2.2 ± 0.2	633 ± 27
54	45.5 ± 0.9	1.8 ± 0.1	569 ± 19
	GROUP III		
5	43.6 ± 2.6	8.0 ± 0.9	1097 ± 101
12	38.3 ± 1.5	9.4 ± 0.8	1166 ± 100
19	37.8 ± 1.6	8.2 ± 0.6	1166 ± 101
26	38.4 ± 1.5	8.7 ± 1.0	1207 ± 82
33	37.3 ± 1.2	7.9 ± 0.7	1129 ± 93
40	36.3 ± 1.5	5.9 ± 0.7	1117 ± 87
47	36.6 ± 1.6	5.8 ± 0.9	1206 ± 84
54	40.3 ± 1.4	4.5 ± 0.8	1042 ± 66

APPENDIX 11

Mean daily DOM intakes (6-15 days prepartum) and lamb birth-weights for ewes in Groups II and III of Glensaugh experiment (cf. Figure 15)

Group II			Group III		
Sheep No	Mean daily intake (g DOM/kg)	Birth-weight (g/kg)	Sheep No	Mean daily intake (g DOM/kg)	Birth-weight (g/kg)
AA	14.2	127	BA	5.1	53
AB	19.4	146	BB	12.6	150
AC	17.2	131	BC	6.4	71
AE	14.1	108	BE	15.1	140
AH	9.4	74	BH	13.8	145
AI	15.0	86	BI	8.2	84
AK	13.5	73	BK	10.6	74
AM	14.6	117	BL	7.5	74
AN	7.6	68	BM	8.0	75
AO	14.6	136	BN	8.4	104
AP	9.1	70	BO	10.6	98
AR	16.1	110	BP	11.4	96
AS	11.1	89	BR	12.7	113
AT	13.3	89	BS	10.8	111
AU	15.9	147	BT	8.4	80
AV	13.7	105	BV	10.1	86
AW	15.1	102	BW	14.0	131
AX	19.5	174			
AY	19.2	102			

X - PUBLICATIONS

Some of the experimental work reported in this thesis has been published in the undernoted papers:

RUSSEL, A. J. F., DONEY, J. M. and REID, R. L. 1967. The use of biochemical parameters in controlling nutritional state in pregnant ewes, and the effect of undernourishment during pregnancy on lamb birth-weight. J. agric. Sci., Camb. 68 : 351-358.

RUSSEL, A. J. F., DONEY, J. M. and REID, R. L. 1967. Energy requirements of the pregnant ewe. J. agric. Sci. Camb., 68 : 359-363.

RUSSEL, A. J. F., GUNN, R. G. and DONEY, J. M. 1968. Components of weight loss in pregnant hill ewes during winter. Anim. Prod. 10 : 43-51.